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Ultraviolet-B Radiation : Effects on Pollen of 34 Taxa, and Inheritance Patterns and Carryover of Radiation Response in Arabidopsis

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ULTRAVIOLET-B RADIATION: EFFECTS ON POLLEN OF 34 TAXA, AND INHERITANCE PATTERNS AND CARRYOVER OF RADIATION RESPONSE IN *ARABIDOPSIS*

by

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ABSTRACT

Ultraviolet-B Radiation: Effects on Pollen of 34 Taxa, and Inheritance Patterns and Carryover of Radiation Response in *Arabidopsis*

by

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Although considerable research has addressed effects of elevated ultraviolet-B (UV-B) radiation on vegetative plant structures and processes, the reproductive biology and patterns of inheritance of UV-B tolerance have received much less attention. I examined the effects of UV-B radiation on pollen of 34 taxa. I also addressed questions concerning the patterns of inheritance of UV-B tolerance in *Arabidopsis thaliana* and examined potential cumulative carryover effects of UV-B exposure through multiple generations of this species.

In the first study, a significant reduction in pollen germination occurred in only five species, but pollen tube growth in more than half of the species exhibited significant reductions. Proportionate to their numbers in this survey of

34 taxa: Monocotyledonous species were more sensitive to UV-B than the dicotyledonous species, wild species were more sensitive than cultivated species, and pollen from plants growing in the field was somewhat more sensitive than pollen from plants grown in the greenhouse. The results also suggested a possible adaptation to UV-B radiation during the course of the season.

The second study probed patterns of inheritance of UV-B tolerance. General combining ability and specific combining ability were both significant for several traits in *Arabidopsis.* This suggests that plant breeders may develop UV-B tolerant strains of plant species both in the form of pure lines and hybrids.

In the third experiment, I found that a significant carryover effect of UV-B exposure through multiple generations *Arabidopsis thaliana* was evident in a subsequent generation, in either the presence or absence of UV-B, once differences in the reactions of different ecotypes were taken into account. However, instead of an expected accumulation of UV-B effect as a greater number of generations were exposed to the radiation, there was an apparent reversion of the cumulative, carryover effect. This was evident in that plants exposed to seven generations of UV-B exhibited much less carryover effect than did plants exposed for only three generations.

(176 pages)

DEDICATION

To my mother for her countless sacrifices

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Javad Torabinejad

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

As stratospheric ozone is depleted, ultraviolet-B (UV-B) radiation that reaches the earth's surface increases (Kerr & McElroy 1993; Madronich *et al.* 1995), and this may lead to changes in biological systems. Higher plant responses elicited by UV-B may involve both vegetative and reproductive structures and processes. Various morphological, physiological, and molecular responses to UV-B radiation have been observed in plants (Strid *et al.* 1994; Bornman & Sundby-Emanuelsson 1995; Björn 1996; Britt 1996; Greenberg *et al.* 1997; McLeod & Newsham 1997; Rozema *et al.* 1997a; Caldwell *et al.* 1998; Jansen *et al.* 1998; Vonarx *et al.* 1998). These changes include a reduction in photosynthesis in some species under some circumstances, but often not under field conditions (Teramura & Sullivan 1994; Björn 1996; Allen *et al.* 1998, 1999), changes in leaf pigmentation (Sisson & Caldwell 1976; Vu *et al.* 1984; Strid & Porra 1992), and foliage anatomy (Cen & Bornman 1990; Nagel *et al.* 1998). In many species leaf expansion and/or stem growth can be reduced (Caldwell & Flint 1994; Johanson *et al.* 1995b; Tosserams & Rozema 1995; Rozema *et al.* 1997b; Deckmyn & Impens 1998). The UV-B radiation can reduce biomass accumulation in certain cultivars or species (Teramura & Sullivan 1987, 1994; Sullivan & Teramura 1992; Deckmyn & Impens 1997). Yet, a number of morphological responses such as increased branching, greater leaf thickness (Tevini & Teramura 1989; Cen & Bornman 1990; Day *et*

al. 1992; Caldwell & Flint 1994; Teramura & Sullivan 1994; Gwynn-Jones *et al.* 1997; Rozema *et al.* 1997a), reduced leaf length, and increased leaf production can occur without subsequent decreases in plant production. Often there can be changes in the balance of interspecific competition (Gold & Caldwell 1983; Barnes *et al.* 1988; Caldwell 1997).

Much of the UV-B radiation research on plants has concentrated on vegetative plant parts and only a small fraction has dealt with reproductive systems. Reports suggest that UV-B may inhibit pollen germination (Campbell *et al.* 1975; Chang & Campbell 1976; Flint & Caldwell 1984) and reduce pollen tube growth (Chang & Campbell 1976; Pfahler 1981). Other reports indicate that UV-B treatment of plants before anthesis can, by some unknown mechanism, substantially affect pollen performance (Musil & Wand 1993; Musil 1995). *In vivo* studies of pollen exposure to UV-B have also demonstrated effects on pollen (Demchik & Day 1996; Feldheim & Conner 1996; Musil 1996; Sampson & Cane 1998). Other environmental stresses such as ozone pollution may interact with UV-B. For example, a reduction in pollen tube growth occurred in the presence of UV-B and ozone (Feder & Shrier 1990); when the two stresses were combined, there was an apparently additive effect of the two stresses on tube growth.

Each of the foregoing investigations was limited to only a few species and radiation levels, and other conditions differed among the studies. Moreover, in most of the *in vitro* experiments in which pollen was exposed to

UV radiation, a very low level of visible radiation was used. Because of these deficiencies, I conducted the experiments presented in Chapter 2, where the results of an extensive survey of pollen from 34 taxa exposed to UV-B radiation are reported. To our knowledge, this study is the first to examine a large number of species under comparable conditions and UV-B flux rates. The objective of this study was to determine patterns of radiation sensitivity by exposing pollen from a number of genera to two UV-B flux rates.

Considerable variation in UV-B responses exists among species (Kossuth & Biggs 1981; Barnes *et al.* 1990; Sullivan *et al.* 1992; Day 1993; McLeod & Newsham 1997; Torabinejad *et al.* 1998) and among varieties within the same species (Teramura 1983; Tevini & Teramura 1989; Ziska *et al.* 1992; Barnes *et al.* 1993; Corlett *et al.* 1997; Correia *et al.* 1998). Genotypic differences were observed in the response of *Arabidopsis thaliana* to UV-B radiation (Jalilova *et al.* 1993; Shishkin & Ivanishchev 1997; Usmanov *et al.* 1988). These intraspecific variations in responsiveness to UV-B suggest that breeding for UV-B tolerance in agricultural species should be feasible (Caldwell *et al.* 1995). Few studies have investigated the inheritance of UV-B tolerance, and none of them have employed a diallel crossing system.

In Chapter 3, I provide information concerning the inheritance of UV-B radiation tolerance by employing *A. thaliana* as a model species. My objectives were to: (1) estimate variability in UV-B tolerance among seven ecotypes of *A. thaliana* and their F1 hybrids to gain an understanding of the genetics of UV-B

radiation sensitivity in this species, (2) determine the importance of general and specific combining abilities (GCA and SCA, respectively) in the inheritance of UV-B responsiveness in *Arabidopsis* with a diallel mating design, and (3) assess the feasibility of developing genotypes that would be less responsive to elevated UV-B radiation levels either from the original parental types or from their hybrids.

Experiments involving a single generation of plants, usually in a single growing season, have demonstrated a variety of responses to UV-B radiation. However, much less is known about potential cumulative carryover effects of UV-B radiation exposure in subsequent growing seasons of perennial plants or in subsequent generations of annual plants. Sullivan and Teramura (1992) and Johanson *et al.* (1995a) speculated that the effects of UV-B radiation might be cumulative for woody perennial species. Cumulative effects were shown for successive generations of an annual desert species (Musil 1996; Midgley *et al.* 1998; Musil *et al.* 1999). Chapter 4 presents a study that was designed to test whether UV-B exposure in earlier generations might be apparent in later generations for three ecotypes of *Arabidopsis*, both in the presence and absence of UV-B. The degree to which these carryover effects might accumulate when a greater number of generations were exposed to UV-B was tested by comparing plants after three and seven generations of exposure to UV-B.

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CHAPTER 2

SUSCEPTIBILITY OF POLLEN TO UV-B RADIATION: AN ASSAY OF 34 TAXA[1]

ABSTRACT

Much of the ultraviolet-B (UV-B) radiation research on plants has concentrated on vegetative plant parts, and only a small fraction has dealt with reproductive systems. Pollen grains of 34 taxa were allowed to germinate and grow under two levels of UV-B radiation (187 and 460 mW/m^2) or no UV-B (control group). Visible radiation at 260 $\mu mol.m^{-2}.s^{-1}$ was present in all treatments. Taxa included those with binucleate and trinucleate pollen types. We detected differences among species. A significant reduction in pollen germination occurred in only five species. Pollen tubes of > 50% of the species showed significant reduction in length. Trinucleate pollen types were more likely to exhibit tube length reduction than the binucleate types. Proportionately more monocotyledonous species were sensitive to UV-B treatment than dicotyledonous species, and proportionately more wild species were sensitive than cultivated species, and pollen collected from plants growing in the field

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[1]Coauthored by Javad Torabinejad, Martyn M. Caldwell, Stephan D. Flint, and Susan Durham; this chapter was previously published in 1998 in American Journal of Botany (85:360-369). Botanical Society of America, Inc. retains full copyrights to the article. See Appendix A of this dissertation for their "permission to reprint" letter. Appendix A also includes release letters of the two coauthors who are not on the supervisory committee.

were somewhat more sensitive than pollen collected from plants grown in the greenhouse. Species in which pollination occurred earlier in the season were more likely to be susceptible to UV-B radiation than those for which anthesis took place later in the season, suggesting a possible adaptation to UV-B radiation.

INTRODUCTION

During the past few decades, the stratospheric ozone reduction problem has stimulated considerable research on higher plant responses to ultraviolet-B (UV-B) radiation (Caldwell and Flint, 1994). However, little of this work has addressed the reproductive biology of plants.

Most of the studies concerning UV-B radiation on reproductive biology have focused on pollen. Pollen can be categorized as having two or three nuclei when released; the number of nuclei tends to follow phylogenetic lines (Brewbaker, 1967). Flint and Caldwell (1986) speculated that binucleate pollen grains, which are less physiologically advanced and slower to germinate, might be more sensitive to UV-B than trinucleate pollen.

The germination of binucleate pollen is often inhibited when placed on artificial media under UV-B radiation. Campbell, Caldwell, and Sisson (1975) reported pollen germination inhibition by UV-B radiation in *Tradescantia (T. subacaulis* X *T. hirsutiflora*), *Petunia hybrida*, and *Vicia villosa*. Chang and Campbell (1976) observed a reduction of pollen germination following UV-B

irradiation of two interspecific *Tradescantia* hybrids (*T. subacaulis* X *T. hirsutiflora* and *T. occidentalis* X *T. ohiensis*) flowers. Flint and Caldwell (1984) exposed pollen grains of the temperate-latitude species (*Papaver rhoeas*, *Cleome lutea*, and *Scrophularia peregrina*) to UV-B radiation equal to maximum levels found in equatorial alpine locations and showed that pollen germination was partially inhibited. Zelles (1974) and Seibold, Zelles, and Ernst (1979) demonstrated both stimulating and inhibiting effects of UV radiation (270-300 nm) on pine pollen tubes. Pine has binucleate pollen (Coulter and Chamberlain, 1917).

Relatively few experiments have evaluated trinucleate pollen because of the difficulty of germinating it on artificial media. Pfahler (1981) found little or no effect on germination or the number of pollen grains that ruptured when maize pollen was treated with UV. Of the four species examined by Flint and Caldwell (1984), the least inhibition of germination occurred in the only trinucleate species used, *Geranium viscosissimum*.

Pollen tube growth under UV-B irradiation has received little attention. A reduction in maize pollen tube growth occurred when germinating pollen grains were exposed to UV-B radiation (Pfahler, 1981). When mature *Zea mays* pollen grains were exposed to UV radiation (wavelength range not specified) before pollination, there was an increased proportion of shrunken kernels in the fruit (Pfahler and Linskens, 1977). Chang and Campbell (1976) observed a reduction of pollen tube growth following UV-B irradiation of flowers of the two

interspecific *Tradescantia* hybrids mentioned above.

Generally, the plant's female reproductive system and developing pollen grains are considered to be well protected from UV-B (Martin, 1970; Flint and Caldwell, 1983). However, recent work showed that UV-B treatment of plants before anthesis can, by some unknown mechanism, have a substantial effect on the performance of pollen. Binucleate Ericaceae species were grown in the greenhouse under different levels of UV-B radiation and at anthesis, their pollen grains were cultured on artificial media (Musil and Wand, 1993). Pollen germination was reduced in *Erica fairii* and *E. curvirostris*, and there was a reduction in pollen tube length of *E. curvirostris*. Musil (1995) exposed *Ixia viridiflora*, *Gladiolus carneus*, *Geissorhiza radians*, and *Babiana rubrocyanea* (all monocots with binucleate pollen type) and *Senecio elegans*, *Pentzia suffruticosa*, *Ursinia anthmoides*, and *Dimorphotheca sinuata* (all dicots with trinucleate pollen type) plants to UV-B and subsequently assayed pollen for germination and tube growth without further exposing the pollen to the UV-B. Pollen tube growth of all four dicots and of one monocot was reduced. Pollen germination of two of the dicot species declined as well. On the other hand, an increase in both pollen tube length and pollen germination occurred in one of the monocot species.

In a greenhouse study, Demchik and Day (1996) evaluated the in vivo reproductive performance of a trinucleate species (*Brassica rapa*) under three levels of UV-B radiation (ranging from that approximating ambient solar UV-B

on 12 March at 39° N to twice ambient levels). They concluded that UV-B radiation higher than ambient levels can reduce viable pollen production, possibly resulting in a substantial limitation in pollination, fertilization, and seed production of natural populations of *B. rapa*. Feldheim and Conner (1996) studied the effects of UV-B on fitness in both *B. rapa* and *B. nigra*. In contrast to Demchik and Day (1996), the authors concluded that although UV-B two to three times greater than ambient solar UV-B (on 1 June at 40° N) was generally detrimental to growth and flowering in both species, total seed production was enhanced at these higher UV-B doses in 75% of the combinations of dose/species they used. They also reported little effect on pollination success or offspring quality. Similar results were obtained for two binucleate species, *Phacelia campanularia* and *Limnanthes alba* (Sampson and Cane, personal communication). Higher UV-B dosages resulted in less successful flowering in *L. alba*. The onset of flowering and the number of flowers, however, were not affected by increased UV-B in plants that did manage to flower. On the other hand, individuals of *P. campanularia* did not differ in their probability of flowering under various levels of UV-B, but flowering was delayed by 1.5 d and lifetime flower production decreased by 4.5% for each 1 $kJ.m^{-2}.d^{-1}$ (biologically effective UV-B) increase in the UV-B dosage. Pollen production of neither species was affected by UV-B.

Musil (1996) studied the effects of UV-B radiation over two generations of *Dimorphotheca sinuata* (trinucleate) to determine whether the UV-B effect on

pollen germination and tube growth would accumulate from one generation to the next. Two populations of *D. sinuata* were exposed either to a UV-B level approximating ambient solar UV-B or to a level greater than ambient UV-B radiation. While pollen germination was significantly reduced under the higher UV-B flux rate, tube length was not affected. On the other hand, there was a significant difference in tube length between the cultivars previously exposed to high- and low-UV-B when tested, indicating an accumulated effect of UV-B. This cumulative effect of UV-B, however, was not observed for pollen germination.

Other environmental stresses may interact with UV-B. *Nicotiana tabacum* and *Petunia hybrida* pollen (both binucleate) were exposed to UV-B, ozone fumigation, and a combination of the two factors (Feder and Shrier, 1990). Both UV-B and ozone reduced pollen tube growth in the two species, and when the two stresses were combined, the reduction of pollen tube growth was more severe than with either stress alone; this appeared to be an additive effect.

Previous research indicates that pollen can be sensitive to UV-B radiation. However, each of the foregoing investigations was limited to only a few species and radiation levels, and other conditions differed among the studies. Moreover, in most of the in vitro experiments where pollen itself was exposed to UV radiation, a very low level of visible radiation was used. To our knowledge, our study is the first to examine a large number of species under

comparable conditions and UV-B flux rates. The objective of our study was to determine patterns of radiation sensitivity by exposing pollen from a number of genera to two UV-B flux rates. Several criteria were used to select species in the survey. Only species with chasmogamous flowers were used. Most of the species typically experience anthesis in the morning or midday hours so that pollen is usually exposed to sunlight during times of the day when the UV-B is appreciable; however, a few species that undergo nocturnal anthesis were intentionally included. The species surveyed included binucleate and trinucleate pollen based on the cytological classification of Brewbaker (1967). The taxa chosen allowed comparison of radiation susceptibility of pollen among different classifications: binucleate and trinucleate pollen, monocot and dicot species, greenhouse- or field-grown plants, and cultivated and wild taxa.

MATERIALS AND METHODS

Plant material--A total of 34 taxa was selected (Table 1). The plant material was either grown in a temperature-controlled greenhouse (provided with supplementary light during short days) or was collected from plants growing in several habitats near Logan, Utah (41° 45' N, elevation of 1500 m). Cultivated plants were collected on or near the Utah State University campus in Logan, UT.

Growth-chamber conditions--All trials were conducted in the same growth chamber. Only one taxon was used in each trial, and two trials were

conducted for each taxon. Within the growth chamber, I provided two levels of biologically effective UV-B radiation (187 and 460 mW/m^2) using a combination of fluorescent sunlamps (Q Panel UV-B 313, Cleveland, OH) and a 6000-W xenon lamp (Atlas Electric, Chicago, IL). Biologically effective UV-B radiation is calculated by weighting the spectral irradiance with a generalized plant action spectrum (Caldwell, 1971) normalized to 300 nm. The higher UV-B flux rate corresponds to solar noon UV-B on 21 June at 41° 45' N, 1500 m (Logan, UT) with a 15% ozone reduction from normal levels. The lower UV-B flux rate corresponds to the normal solar UV-B received between 0900 and 0930 (solar time) on 21 June for the same location. The flux rates were calculated using the Green, Cross, and Smith (1980) model. The desired flux rates were obtained by masking portions of the fluorescent lamp tubes with aluminum foil. In addition to UV-B radiation, visible radiation (260 $\mu mol.m^{-2}.s^{-1}$) also was provided by the xenon arc lamp. A total of 14.4 $\mu mol.m^{-2}.s^{-1}$ of ultraviolet-A was measured in the radiation received from both the fluorescent sunlamps and the xenon lamp. The UV flux was measured with a double-monochromator spectroradiometer with holographic gratings (Optronic Model 742, Orlando, FL) calibrated against a 1000-W tungsten-halogen standard lamp from the National Institute of Standards and Technology. Wavelength accuracy was checked before this calibration and again when the instrument was moved to the growth chamber by scanning a low-pressure mercury lamp with a discharge at distinct, well-separated, known emission lines. The spectroradiometer and a quantum

sensor (LI-COR, Lincoln, NE) to measure visible radiation were used to locate four sites in the growth chamber that received the two desired flux rates of UV-B and the same amount of visible radiation.

One pollen culture chamber (PCC) was placed at each selected site. The PCC was a two-celled, plexiglass chamber in which the pollen was held for irradiation. The two cells of the chamber were equipped with filters to either transmit or absorb UV-B. One cell of the PCC was covered with a clear cellulose acetate film (0.13 mm thick), which transmitted the UV-B radiation (treatment), and the other cell with polyester (Mylar) film (0.13 mm thick) to absorb UV-B radiation (control). The chambers allowed ventilation under the filters to prevent condensation, provide humidity, and regulate temperature in the cells. The air temperature in the growth chamber was maintained at 24°C with dewpoint ranging between 18° and 20°C. Air exchange in the PCCs was ~2 L/min. Because pollen germination and pollen tube growth are considerably influenced by temperature and humidity, we conducted bioassays to insure that conditions in the cells of the PCC were identical using pollen of *Tradescantia blossfeldiana* and *Zea mays*. To conduct the bioassay, the fluorescent sunlamps were turned off and the UV-B radiation emitted from the xenon lamp was filtered out by a sheet of Mylar. The tests were conducted twice for each species and showed no significant differences in pollen germination and tube growth in the test cells.

Pollen culture--For all trials, we obtained pollen from newly opened

flowers, transferred the pollen with a camel-hair brush, and cultured the pollen on a microscope slide coated with an agar-based medium. Three basic media (Brewbaker and Kwack, 1963; Bar-shalom and Mattsson, 1977; Pfahler, 1981) were used to accommodate growth preferences of the different species. The coated slides were placed inside the PCCs under the filters. The PCCs were then transferred to the growth chamber, and the pollen was exposed to radiation until most of the pollen grains had germinated. Pollen grains were considered germinated and used for pollen tube measurements if the pollen tubes were longer than the diameter of the pollen grains.

Because pollen germination and pollen tube growth rates differ among taxa, the time of radiation exposure differed among species (Table 1). After radiation exposure, the PCCs were removed, and the pollen was preserved in Farmer's fixative (3:1 ratio of ethanol to acetic acid). A coverslip was placed over the pollen grains on the microscope slides, and each slide was placed in a petri dish at 4°C until it could be analyzed for pollen germination and pollen tube length under a light microscope. An average of 41 pollen tubes per treatment was measured for each species. Percentage germination and mean pollen tube length were computed for each cell.

Experimental design and analysis of data--For both response variables (pollen tube length and percentage germination) observed in each PCC, we computed percentage change relative to control as

$$[\frac{C-T}{C}]x100\%$$

where *C* is control (i.e., the UV-B blocking filter) and *T* is treatment (i.e., the UV-B transmitting filter). Data were analyzed on their original scales.

In each trial, two PCCs were irradiated at each of the two levels of UV-B. These two PCCs were considered to be subsamples, and prior to analysis, the mean relative percentage change over the two subsamples was computed for each response variable and was used as the data in the analysis. Each response variable was analyzed separately in an analysis of variance of a one-way factorial in a randomized block design. Trial was the block factor. The experimental factor was UV-B radiation flux at two levels (high and low). The effect of treatment (filter-type) was evaluated by testing whether the model intercept was equal to zero. Data were analyzed using the GLM procedure in SAS Release 6.11 (SAS, 1988). Two species were not analyzed because of lack of replication. See Appendix B (Tables B.1-B.59) for a complete list of ANOVA tables.

RESULTS

Pollen tube growth of many taxa exhibited considerable sensitivity to UV-B (Fig. 1). There was a significant ($P < 0.10$) reduction in pollen tube length of > 50% of the species. All monocotyledonous taxa (regardless of pollen type)

suffered reductions in pollen tube length when exposed to either UV-B flux rate (Fig. 1A) except for *Allium ostrowskianum*, where an effect was only apparent under the higher UV-B radiation level. The tube length reduction was significant in five of seven monocot species.

Most members of the binucleate Rosaceae also responded to UV-B radiation with a significant reduction in tube length under both flux rates of UV-B radiation (Fig. 1B). We observed variable responses within the pairs of species from the Caprifoliaceae, Papaveraceae, and Solanaceae (Fig. 1C). Significant tube length reductions occurred in two of the three Onagraceae species (Fig. 1D). The greater UV-B flux rate appeared to lead to more inhibition of tube elongation than the lower UV-B level (Fig. 1). Significant differences, however, were observed in only five cases.

Of the trinucleate species, five of seven (71%) showed significant inhibition of pollen tube elongation (at $P < 0.10$) compared to 15 of 25 (60%) of the binucleate species (Fig. 2A). The same proportions (71 and 60%) of monocots and dicots, respectively, were inhibited (Fig. 2B). A significant tube length reduction occurred in nine of 12 (75%) of field-grown species compared to 11 of 20 (55%) of greenhouse-grown taxa (Fig. 2C). A similar comparison showed six of nine (66%) wild species showing inhibition compared to 14 of 23 (61%) cultivated species (Fig. 2D).

Field-collected pollen of species whose anthesis occurred earlier in the spring tended to suffer greater inhibition of tube elongation than those with

anthesis closer to the solstice (Fig. 3).

Although there was a tendency for reduction in pollen germination in many of the taxa, a significant reduction ($P < 0.1$) occurred in only five species (Table 2). All of these species have binucleate pollen. Pollen germination was reduced in *Oenothera caespitosa* and *O. pallida*, both of which belong to the Onagraceae. The third member of this family (*Epilobium* sp.), however, did not exhibit a reduction in pollen germination ($P = 0.21$).

DISCUSSION

The lower UV-B flux rate employed in these experiments (187 mW/m^2 biologically effective radiation) was sufficient to cause reduced pollen tube growth in at least 50% of the species tested. This flux level of solar UV-B is normally met or exceeded between 0900 and 1500 on 21 June for Logan, Utah. At noon, the flux normally reaches 347 mW/m^2. Most of the species used in this survey were chosen because they typically release pollen in the morning or midday hours. Thus, the lower flux rate employed in this experiment is within a range realistically expected to cause some damage to developing pollen tubes if the duration of exposure is sufficient.

The total exposure times in our trials (Table 1) are for the entire pollen sample and do not represent the duration of exposure for individual pollen tubes. For an individual pollen tube, exposure was probably much shorter than the irradiation times shown in Table 1. The duration of exposure of individual

pollen tubes to appreciable UV-B flux rates in nature depends on the time required for individual pollen tubes to grow from the germinated pollen grain to penetration of the stigma; this time varies considerably among species (Heslop-Harrison, Heslop-Harrison, and Barber, 1975; Heslop-Harrison, 1980; Ferrari et al., 1983; Chichirricco and Caiola, 1986). Published observations reveal a wide range of times required for individual pollen grains to germinate and pollen tubes to grow and penetrate the style (Table 3). These times are based on in vivo experiments with intact plants or with intact flowers and are probably the most relevant data, since pollen development in the milieu of the stigma cannot be duplicated with artificial media (Roberts et al., 1983; Mulcahy and Mulcahy, 1985). Roberts et al. (1980, cited by Roberts et al., 1983) compared the in vitro and in vivo hydration rates in *Brassica oleracea* (a trinucleate); it took only 6 s for this process to be completed on the liquid medium while it lasted > 1 h on the stigmatic surface. Cresti, Ciampolini, and Sarfatti (1980) compared pollen germination and tube organization of *Lycopersicum peruvianum* (a binucleate) in vivo and in vitro. Morphological changes occurring during activation and germination were similar in the two situations, but the speed of development was different. While in vitro hydration occurs immediately after seeding, this process takes 15 min to complete in vivo. Pollen tube emission was about five times faster in the in vitro environment. Heslop-Harrison (1980) observed the same situation in *Secale cereale* (a trinucleate). Thus, our UV-B experiments, which necessarily had to

be conducted with pollen in artificial media, may represent a conservative estimate of pollen exposure to radiation. In sunlight, the exposure during the middle hours of the day would seem to be sufficient to result in some inhibition unless there are other mitigating factors as discussed later.

Pollen grain and tube damage may be due to several factors, including pollen tube membrane lesions resulting from the direct effects of UV-B radiation on membrane lipids or proteins (Wright, Murphy, and Travis, 1980; Imbrie and Murphy, 1982; Kramer et al., 1991; Predieri et al., 1995). DNA lesions may also be involved (Setlow, 1966; Caldwell, 1971). Stapleton and Walbot (1994) showed that flavonoids may be synthesized as a result of the UV-elicited membrane changes (Murphy, 1983) and may contribute to protecting membranes and DNA from radiation damage. Specific enzyme systems for repairing DNA damage have also been shown to exist in pollen grains. Ikenaga, Kondo, and Fujii (1974) isolated photolyase, the enzyme responsible for photorepair of DNA lesions, from dormant maize pollen, and Jackson and Linskens (1979) have shown that photoreactivation and dark repair processes operate in UV-irradiated *Petunia* pollen in early stages of pollen germination. Two photorepair systems exist in plants that repair two major types of lesions, cyclobutane pyrimidine dimers and pyrimidine (6-4) pyrimidinone dimers (Chen, Mitchell, and Britt, 1994). In other plant tissues, both of these photorepair systems were shown to be effective at visible radiation fluxes that are only ~5% of midday sunlight in midsummer (Chen,

Mitchell, and Britt, 1994; Takeuchi et al., 1996). Thus, if DNA photorepair is the primary mechanism of amelioration of UV-B damage in pollen, the visible radiation employed with the UV-B in our experiments (260 $\mu mol.m^{-2}.s^{-1}$, 400-700 nm, > 10% of midday sunlight) should have been sufficient to allow these photorepair systems to operate effectively. Most in vitro studies of UV-B effects on pollen have not employed concomitant visible radiation.

Structural characteristics of pollen from different species may contribute to the sensitivity or tolerance of pollen to UV-B radiation. Pollenkitt, which covers the pollen grains of many insect-pollinated plants (Echlin, 1971), is composed of lipoidal material, carotenoids, breakdown products of tapetal proteins, chalcones, and flavonoids (Heslop-Harrison, 1975; de Vlaming and Kho, 1976; Wiermann and Vieth, 1983; Vogt and Taylor, 1995). Among other biological functions, this oily layer may protect pollen from the damaging effects of UV radiation (Linskens, 1964; Echlin, 1971).

Flint and Caldwell (1986) suggested that binucleate pollen grains may be more susceptible to UV-B radiation than trinucleate pollen because trinucleate pollen has many advanced physiological and developmental characteristics compared with binucleate pollen. For example, binucleate pollen germinates more slowly, and the pollen tubes take longer to grow and penetrate the style than trinucleate pollen (Flint and Caldwell, 1986; also Table 3). Slower germination and growth would provide a greater opportunity for solar UV-B radiation to affect pollination by binucleate pollen than would be the case

for trinucleate pollen. However, other factors may counteract the influence of differences in developmental time. Binucleate pollen grains reportedly contain more elutable compounds, including flavonoids, than trinucleate pollen grains (Kirby and Smith, 1974; Wiermann and Vieth, 1983). Furthermore, characteristics of the stigmatic surface may play an important role in protecting the pollen tube. Martin (1970) demonstrated the presence of UV-absorbing compounds in stigmatic exudates. A "wet" stigma has been observed more often in those species having binucleate pollen types. All wet stigma forms within the monocotyledons, surveyed by Heslop-Harrison and Shivanna (1977), have binucleate pollen. However, for dicotyledons only 61% of the wet stigma forms have binucleate pollen and 17% have trinucleate pollen.

Although UV-B radiation tended to reduce pollen germination in many taxa, the reduction was significant in only five species. The small number of significant responses may be due partly to the potentially shorter germination time in artificial media than in vivo. Furthermore, the pollen grains were not exposed to the UV-B radiation while still in the dry state before being placed on the culture medium. In the dry state, pollen may also be susceptible to some damage from UV-B (Torabinejad, personal observation; Demchik and Day, 1996).

Two of the five species with significantly reduced pollen germination by the UV-B radiation normally experience anthesis at night and may, therefore, not be adapted to tolerate sunlight UV-B. All of the species that were

significantly reduced in pollen germination by UV-B were binucleate; however, there were so few species significantly affected that generalizations cannot be made regarding pollen type.

A higher proportion of the trinucleate pollen showed sensitivity to UV-B; ultraviolet-B decreased pollen tube growth in five of seven trinucleate species tested and in 15 of 25 binucleate species tested (Fig. 2A). Due to its faster growth rate, a trinucleate pollen tube should experience less exposure to solar UV. Thus, our study may exaggerate the effect of UV-B on pollen of the trinucleate species. Although pollen tubes of monocots were more susceptible to UV-B radiation than those of dicots (Fig. 2B), pollen germination was not reduced in any of the monocot species. Musil (1995) reported that pollen of monocot species cultured in vitro was more tolerant of UV-B radiation than that of dicots. However, in that study, the pollen-donor plants were irradiated, but not the pollen grains themselves. Pollen tube elongation was more likely to be affected by UV-B radiation in field-grown species than in greenhouse-grown species (Fig. 2C), and in wild species compared to cultivated species (Fig. 2D). For the 14 species whose pollen grains were collected in the field, there was a weak but significant correlation ($r^2 = 0.28$ and $P = 0.05$) between sensitivity of pollen tube elongation to UV-B radiation and time of the year when pollen was collected (Fig. 3). This correlation might suggest some degree of acclimation with increasing insolation as the season progresses.

As discussed earlier, UV-B sensitivity of pollen and pollen tubes can be

affected by several factors, such as exposure of pollen to radiation before germination, the interval of radiation exposure between pollen germination and pollen tube penetration in the stigma, and various shielding and repair mechanisms. Our use of in vitro experiments allowed the testing of several species under comparable conditions. However, caution is warranted in extrapolating to pollen sensitivity in nature, which can be better tested under field conditions with in vivo experiments. However, the difficulty of in vivo experiments necessarily limits the number of species that can be tested.

In summary, we found that (1) pollen tube growth for 19 of 34 taxa was reduced by UV-B radiation. Tube growth for one species was increased by UV-B exposure. (2) Species with trinucleate pollen were more likely to have reduced pollen tube growth than species with binucleate pollen. (3) Monocot species were more likely to exhibit reduced pollen tube growth than dicot species. (4) Field-grown species were more likely to exhibit reduced pollen tube growth than greenhouse-grown species. (5) Wild species were more likely to exhibit reduced pollen tube growth than cultivated species. The difference between the two, however, was not as large as in the previous cases. (6) Increased sensitivity of pollen tubes to UV-B was associated with earlier anthesis dates. (7) While UV-B appeared to reduce in vitro pollen germination in many taxa, the reduction was significant in only five of 28 species.

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Table 1. Taxa and exposure time.

Families	Taxa	Seed source[a]	Exposure time (min)	Pollen type[b]	Subclass[c]
Anacardiaceae	*Pistacia vera* Mill.	1	180	B	D
Begoniaceae	*Begonia semperflorens* Hook.	1	70	B	D
Brassicaceae	*Isatis tinctoria* L.	2	80	T	D
Capparaceae	*Cleome serrulata* Pursh.	2	180	B	D
	C. spinosa Sw. 'Spider Plant'	3	90	B	D
Caprifoliaceae	*Lonicera periclymenum* Sw.	1	165	T	D
	Viburnum lantana L.	1	120	T	D
Caryophyllaceae	*Cerastium tomentosum* Boj.	4	75	T	D
Commelinaceae	*Tradescantia blossfeldiana* Hort. ex Blossfeld. 'Purple Heart'	1	60	B	D
Hydrophyllaceae	*Nemophila maculata* Benth. ex Lindl. 'Five Spot'	5	50	B	D

Table 1. Continued.

Families	Taxa	Seed source	Exposure time (min)	Pollen type	Subclass
	Phacelia tanacetifolia Benth.	6	60	B	D
Liliaceae	*Allium ostrowskianum* Regel.	7	95	B	M
	Lilium sp.	8	165	B	M
	Smilacina stellata Desf.	2	115	B	M
	Zigadenus venenosus Desf.	2	60	B	M
Onagraceae	*Oenothera caespitosa* Gill. ex Hook. & Arn.	2	60	B	D
	O. pallida Lindl. 'White'	9	45	B	D
	Epilobium sp.	8	50	B	D
Papaveraceae	*Eschscholzia californica* Cham. 'Orange'	10	135	B	D
	Papaver somniferum L.	11	60	B	D

Table 1. Continued.

Families	Taxa	Seed source	Exposure time (min)	Pollen type	Subclass
Poaceae	*Secale cereale* L.	2	40	T	M
	Zea mays L. 'Sweet Corn Bodacious Hybrid'	12	90	T	M
	Z. mays 'Popcorn South American Yellow'	12	90	T	M
Rosaceae	*Malus* sp. 'Spring Snow' crabapple	13	150	B	D
	Prunus avium L. 'Lambert'	14	120	B	D
	P. cerasifera Ehrh.	1	105	B	D
	P. cerasus Scop. 'Mt. Morrencey'	14	95	B	D
	P. mahaleb L.	2	105	B	D
	P. virginiana L.	2	120	B	D
	Pyrus communis L.	1	125	B	D

Table 1. Continued.

Families	Taxa	Seed source	Exposure time (min)	Pollen type	Subclass
	Sorbus aucuparia Poir.	1	65	B	D
Solanaceae	*Nicotiana tabacum* L. c.v.'Xanthi'	15	180	B	D
	Petunia hybrida Hort. ex Vilm.'Ultra Pink'	16	105	B	D
Verbenaceae	*Caryopteris clandonensis* Hort.	1	120	T	D

[a] 1. Locally cultivated, 2. Locally collected, 3. W. Altee Burpee @ Co., Warminster, PA, 4.Express Seed Co., Oberlin, OH, 5. Lake Valley Seed Inc., Boulder, CO, 6. Wild Seed Farms Inc., Eagle Lake, TX, 7. Langeveld, Freehold, NJ, 8. Unknown, 9. Comstock Seed, Reno, NV, 10. Northrup King Co., Minneapolis, MN, 11. McCormick and Co. Inc., Hunt Valley, MD, 12. The Chas. H. Lilly Co., Portland, OR, 13. The Greenhouse Inc., Logan, UT, 14. Zollingers fruit farm and nursery, Logan, UT, 15. Oregon State University, Corvallis, OR, 16. Vaughan's Seed Company, Downers Grove, IL.
[b] B: Binucleate Pollen, T: Trinucleate Pollen.
[c] D: Dicot, M: Monocot.

Table 2. Relative change in pollen germination (% of control) and *P*-values for the test of UV-B effect. NA indicates that data did not exist or were insufficient for statistical tests.

	Radiation level		
Species	Low	High	*P*-value
Pistacia vera	-7	+8	0.98
Begonia semperflorens	NA	NA	NA
Isatis tinctoria	NA	NA	NA
Cleome serrulata	NA	NA	NA
C. spinosa	+3	+35	0.26
Lonicera periclymenum	-15	-15	0.17
Viburnum lantana	+110	-21	0.43
Cerastium tomentosum	NA	NA	NA
Tradescantia blossfeldiana	-8	+12	0.80
Nemophila maculata	-22	-3	0.57
Phacelia tanacetifolia	+11	+1	0.14
Allium ostrowskianum	-8	-8	0.25
Lilium sp.	-13	-18	0.19
Smilacina stellata	NA	NA	NA
Zigadenus venenosus	+25	+7	0.22
Oenothera caespitosa	-7	-9	0.02
Oenothera pallida	-8	-12	0.07
Epilobium sp.	-21	-21	0.21

Table 2. Continued.

Species	Radiation level		*P*-value
	Low	High	
Eschscholzia californica	-17	+7	0.86
Papaver somniferum	NA	NA	NA
Secale cereale	+5	-4	0.94
Zea mays (Sweet corn)	-13	+8	0.17
Z. mays (Popcorn)	-2	+1	0.94
Malus sp.	+12	-60	0.38
Prunus avium	-8	+6	0.91
P. cerasifera	-13	-15	0.04
P. cerasus	-14	-2	0.07
P. mahaleb	+9	-5	0.78
P. virginiana	-6	-6	0.48
Pyrus communis	-18	-6	0.09
Sorbus aucuparia	-1	-7	0.13
Nicotiana tabacum	-4	-15	0.48
Petunia hybrida	NA	NA	NA
Caryopteris clandonensis	0	+2	0.80

Table 3. Time (in min) to germination and germination to stigma penetration in bi- and trinucleate pollen grains.

	Pollen type	Range	Mean	Median	# of species	Reference[a]
Germination	Binucleate	5-210	57.0	33.0	12	2-6, 8-11, 13-15,
	Trinucleate	1-180	30.0	10.0	19	17-20, 22-23,
						25-33
Stigma penetration	Binucleate	10-60	37.0	40.0	3	1-2, 6-8, 10, 12,
	Trinucleate	4-30	13.0	9.0	9	16, 20-21, 24

[a] 1. Pope (1937); 2. Brown and Shands (1957); 3. Kroh (1966); 4. Jensen and Fisher (1970); 5. Dickinson and Lewis (1973); 6. Chandra and Bhatnager (1974); 7. Dickinson and Lawson (1975); 8. Heslop-Harrison, Heslop-Harrison, and Barber (1975); 9. Chang and Struckmeyer (1976); 10. Heslop-Harrison (1977); 11. Sedgley (1977); 12. Hoekstra and Bruinsma (1978); 13. Pacini and Sarfatti (1978); 14. Sedgley and Buttrose (1978); 15. Cass and Peteya (1979); 16. Sastri and Shivanna (1979); 17. Herrero and Dickinson (1980); 18. Heslop-Harrison (1980). 19. Wilms (1980); 20. DuBay (1981); 21. Heslop-Harrison and Heslop-Harrison (1981); 22. Ameele (1982); 23. Ghosh and Shivanna (1982); 24. Owens and Horsfield (1982); 25. Pundir, Abbas, and Al-Attar (1983); 26. Sarr, Fraleigh, and Sandmeier (1983); 27. Heslop-Harrison, Roger, and Heslop-Harrison (1984); 28. Russell (1986); 29. Burson (1987); 30. Murdy and Carter (1987); 31. Palser, Rouse, and Williams (1989); 32. Thomson (1989); 33. Marquard (1992).

Fig. 1. Mean percentage change in pollen tube length under two UV-B radiation flux rates (187 and 460 mW/m^2) relative to the control (no UV-B radiation). Numbers below the graph are observed significance levels from the analyses of variance. The numbers in the first row are *P* values for the tests of treatment effect, i.e., whether UV-B radiation affects tube length. The numbers in the second row are *P* values for tests of whether tube length is affected by the flux rate (low vs. high) of UV-B radiation. NA indicates taxa for which no tests of significance were computed.

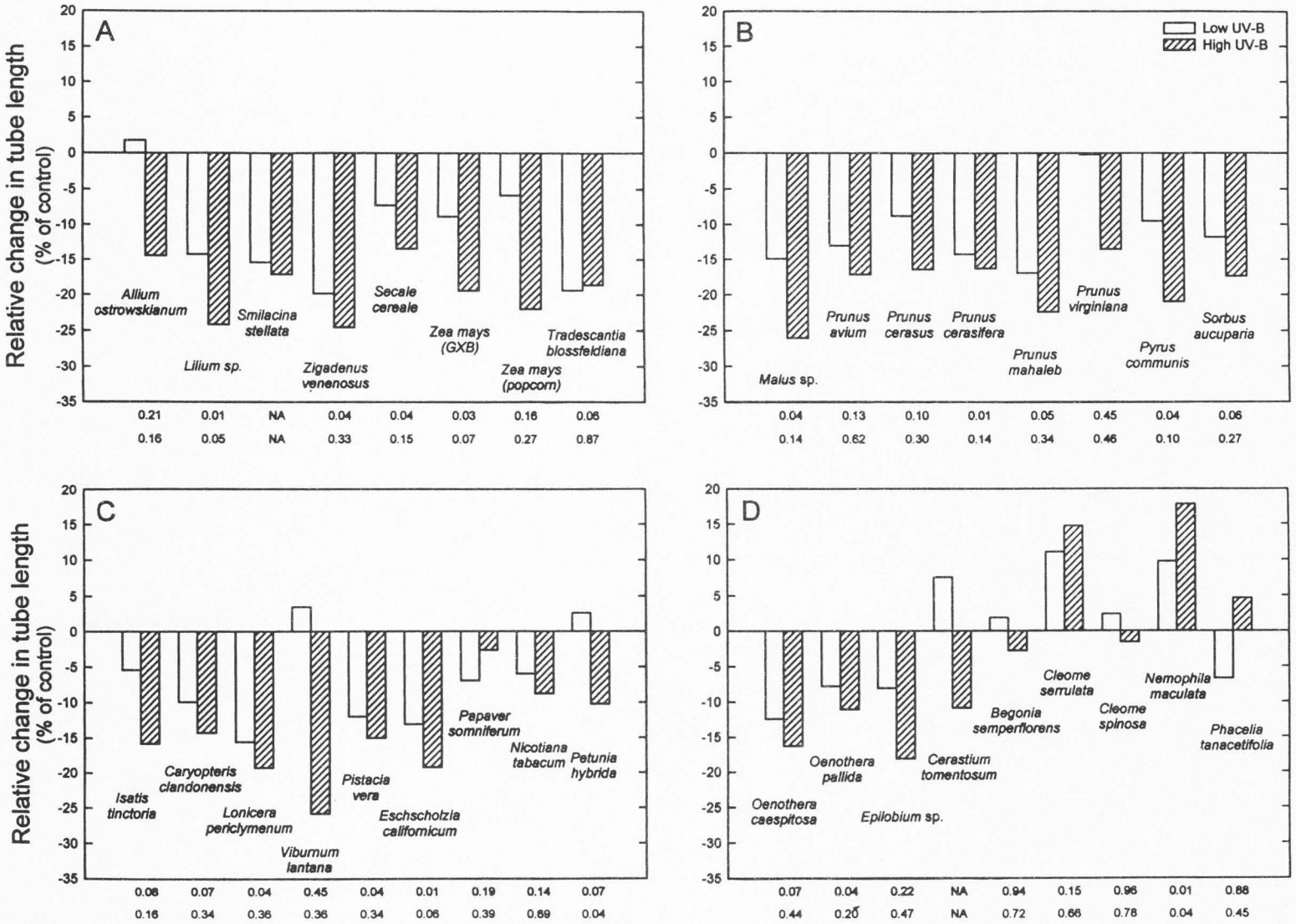
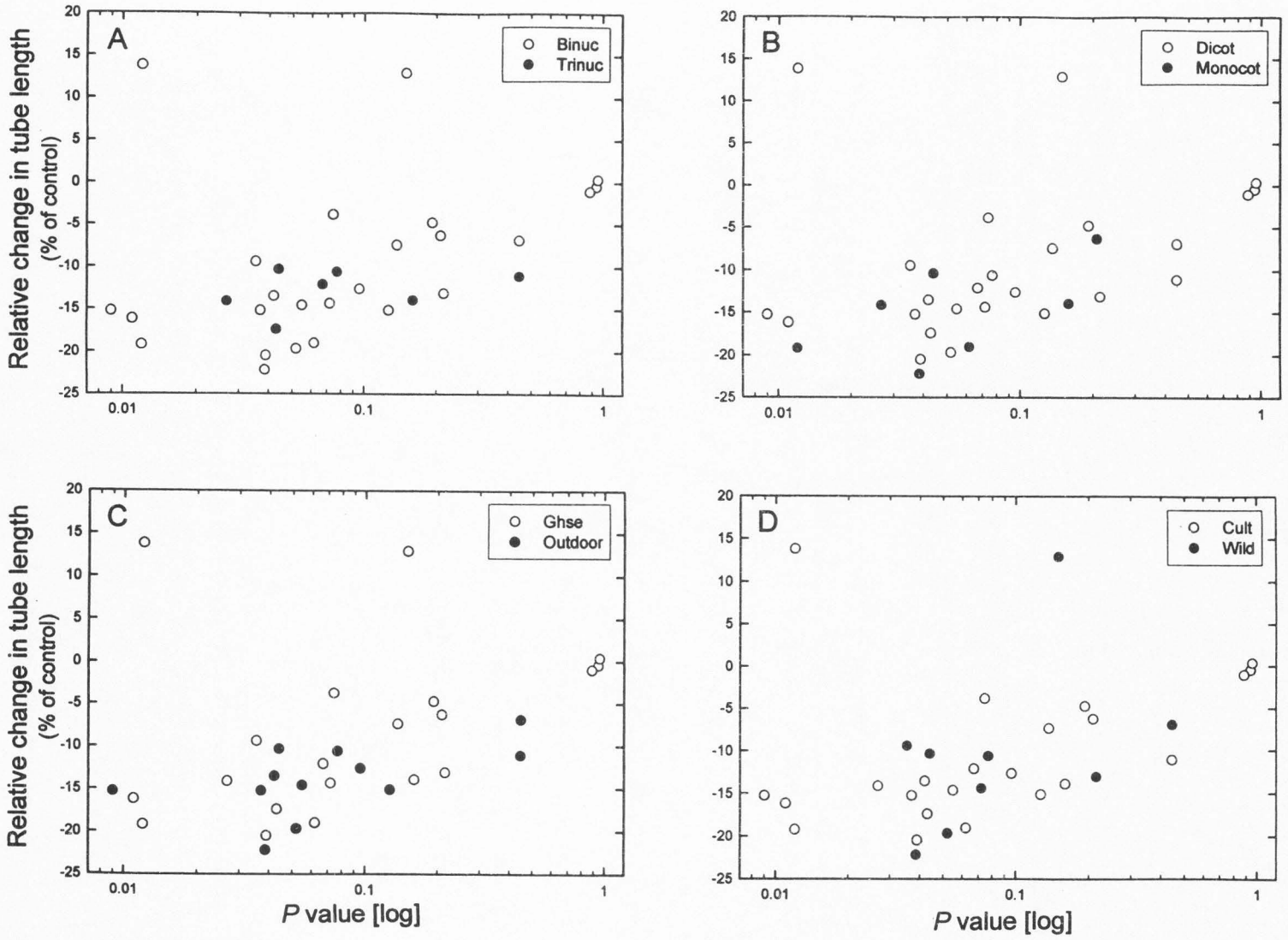

A
Relative change in tube length (% of control)
Allium ostrowskianum
Lilium sp.
Smilacina stellata
Zigadenus venenosus
Secale cereale
Zea mays (GXB)
Zea mays (popcorn)
Tradescantia blossfeldiana
0.21 0.01 NA 0.04 0.04 0.03 0.16 0.06
0.16 0.05 NA 0.33 0.15 0.07 0.27 0.87
B
Low UV-B
High UV-B
Malus sp.
Prunus avium
Prunus cerasus
Prunus cerasifera
Prunus mahaleb
Prunus virginiana
Pyrus communis
Sorbus aucuparia
0.04 0.13 0.10 0.01 0.05 0.45 0.04 0.06
0.14 0.62 0.30 0.14 0.34 0.46 0.10 0.27
C
Relative change in tube length (% of control)
Isatis tinctoria
Caryopteris clandonensis
Lonicera periclymenum
Viburnum lantana
Pistacia vera
Eschscholzia californicum
Papaver somniferum
Nicotiana tabacum
Petunia hybrida
0.06 0.07 0.04 0.45 0.04 0.01 0.19 0.14 0.07
0.16 0.34 0.36 0.36 0.34 0.06 0.39 0.69 0.04
D
Oenothera caespitosa
Oenothera pallida
Epilobium sp.
Cerastium tomentosum
Begonia semperflorens
Cleome serrulata
Cleome spinosa
Nemophila maculata
Phacelia tanacetifolia
0.07 0.04 0.22 NA 0.94 0.15 0.96 0.01 0.88
0.44 0.20 0.47 NA 0.72 0.66 0.78 0.04 0.45

Fig. 2. Mean relative percentage change in pollen tube length under two UV-B radiation flux rates (187 and 460 mW/m^2) relative to the control (no UV-B radiation) for species classified as trinucleate and binucleate (2A), dicots and monocots (2B), greenhouse- and field-grown (2C), and wild and cultivated (2D) vs. observed significance levels for tests of treatment effect, i.e., whether UV-B radiation affects tube length. The values are relative changes averaged over both UV-B radiation levels. A log scale is used to maintain resolution at small values of *P*.


A
Binuc
Trinuc
B
Dicot
Monocot
C
Ghse
Outdoor
D
Cult
Wild
Relative change in tube length (% of control)
P value [log]
20
15
10
5
0
-5
-10
-15
-20
-25
0.01
0.1
1

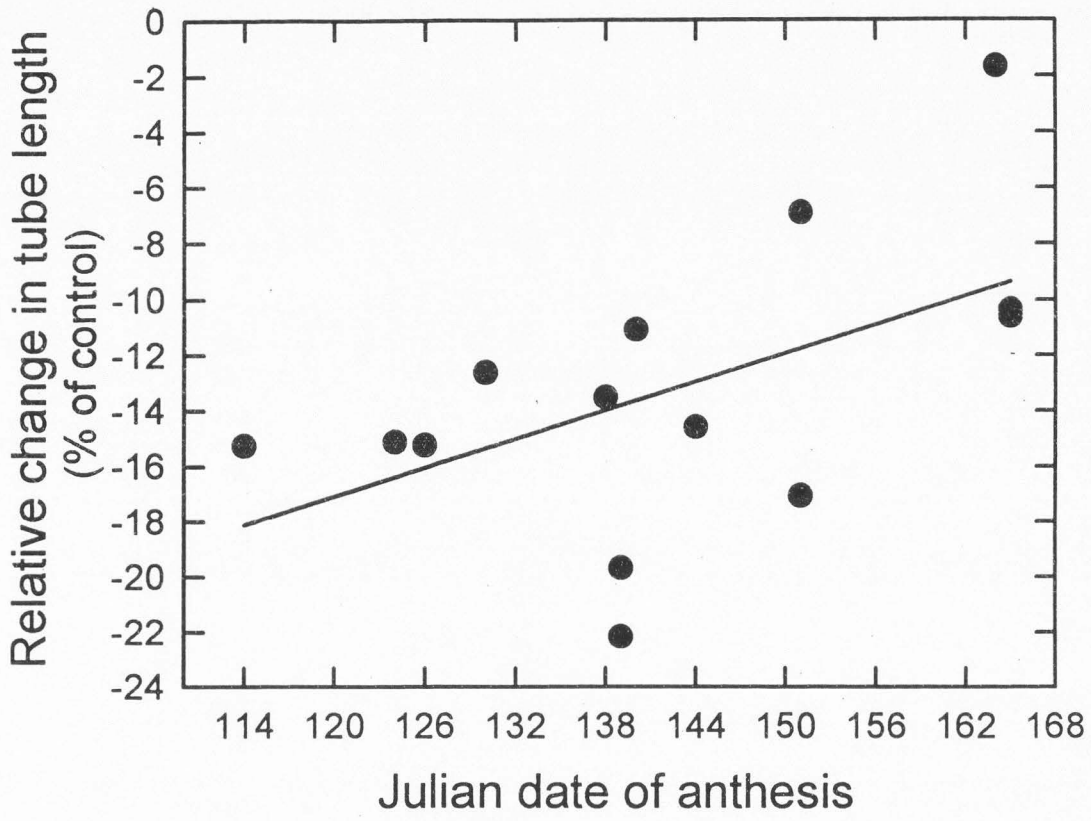


Fig. 3. Mean percentage change in pollen tube length relative to the control in field-grown species at the time of anthesis (in Julian days). The values are relative changes averaged over both UV-B radiation levels.

CHAPTER 3

INHERITANCE OF UV-B TOLERANCE IN SEVEN ECOTYPES OF *ARABIDOPSIS THALIANA* AND THEIR F1 HYBRIDS[1]

Abstract

We used a partial diallel mating design to examine morphological response to supplementary ultraviolet-B (UV-B) radiation of seven ecotypes of *Arabidopsis thaliana* L. Heynh. from several locations in Europe. We were particularly interested in the inheritance of UV-B tolerance by the F1 generation. Morphological traits included plant height, rosette diameter, number of shoots and branches, and reproductive and vegetative dry mass. To effect a large difference in treatments, plants under treatment received 11 kJ m^{-2} d^{-1} of biologically effective UV-B radiation while control plants received no UV-B radiation. Genotype effects were observed for all traits, but a significant treatment effect and genotype X treatment interaction were detected only for plant height, rosette diameter, and reproductive and vegetative dry mass. Treatment effect was marginal for number of shoots. General combining ability was significant ($P < .1$) for plant height and vegetative mass, whereas specific combining ability was significant ($P < .1$) for rosette diameter and vegetative mass. If these results for *Arabidopsis* are applicable to crop species, they

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[1]Coauthored by Javad Torabinejad and Martyn M. Caldwell.

suggest that plant breeders could develop pure lines and hybrids with improved tolerance to UV-B radiation.

Introduction

During the past few decades, the ozone reduction problem has stimulated considerable research on higher plant responses to ultraviolet-B (UV-B) radiation (Caldwell and Flint 1994). Both vegetative and reproductive characteristics have been studied. When exposed to elevated UV-B radiation, higher plants exhibit various physiological and morphological changes (Björn 1996; Caldwell et al. 1998; Greenberg et al. 1997; Jansen et al. 1998; Rozema et al. 1997; Strid et al. 1994), and considerable variation exists both among species (Barnes et al. 1990; Day 1993; Kossuth and Biggs 1981; McLeod and Newsham 1997; Sullivan et al. 1992; Torabinejad et al. 1998) and among varieties within the same species (Corlett et al. 1997; Correia et al. 1998; Teramura 1983; Tevini and Teramura 1989; Ziska et al. 1992). This intraspecific variation in responsiveness to UV-B suggests that breeding for UV-B tolerance in agricultural species should be feasible (Caldwell et al. 1995).

Only few studies have investigated the inheritance of UV-B tolerance, and none of them have employed a diallel crossing system. Sato et al. (1994) observed three distinctive genotypes in F3 plants of rice (*Oryza sativa*): UV-tolerant, sensitive, and segregating heterozygous plants. Sato et al. (1994)

concluded that recessive alleles were important in determining sensitivity of rice to UV-B damage.

Genotypic differences were observed for *A. thaliana* response to UV-B radiation (Jalilova et al. 1993; Shishkin and Ivanishchev 1997; Usmanov et al. 1988). Usmanov et al. (1988) used 19 genetic lines of *Arabidopsis* to determine intraspecific genetic differences for UV-B sensitivity in survival, developmental rate, plant height, and number of pods per plant. They found that these genotypes differed significantly in their response to UV-B, that differences in intraspecific responses to UV-B could be considerable, and that the intraspecific differences exceeded both interspecific and intergeneric differences. There are reports on UV-B sensitivity of *Arabidopsis* mutants designed to probe mechanisms of UV-B tolerance (Britt et al 1993; Chen et al. 1994; Jenkins et al. 1995; Landry et al. 1995; Rao et al. 1996).

The objectives of our study were to (1) estimate variability in UV-B tolerance among seven ecotypes of *A. thaliana* and their F1 hybrids to gain an understanding of the genetics of UV-B sensitivity in this species, (2) determine the importance of general and specific combining abilities (GCA and SCA, respectively) in the inheritance of UV-B responsiveness in *Arabidopsis*, and (3) assess the feasibility of developing genotypes that could tolerate elevated UV-B radiation levels either from the original parental types or from their hybrids. The GCA refers to mean performance of a parental type in a series of hybrid matings and SCA indicates the performance of a particular hybrid

compared to the expected value based on the mean performance of the lines involved (Sprague and Tatum 1942). The GCA represents primarily additive genetic variation and SCA represents nonadditive genetic variation, such as dominance and epistasis.

Materials and Methods

Plant Material and Growth Conditions

Seven ecotypes of *Arabidopsis thaliana* from several locations in Europe were obtained from the *Arabidopsis* Biological Resource Center at Ohio State University. All of the ecotypes came from populations that were within about 100 m of sea level and 3° latitude; therefore, no sizeable differences were expected in their original solar UV environment (Table 4). In an earlier trial, these homozygous genotypes showed variation in sensitivity to UV-B radiation in a temperature-controlled greenhouse in Logan, Utah (41° 45' N, elevation of 1500 m). The flowers were emasculated and subsequently pollinated by pollen from donor plants. After maturation, seeds were collected and used for the diallel experiments.

A few seeds of both parents and their F1 progenies were sown in a medium consisting of equal proportions of vermiculite, bark, peat moss, and perlite. This potting medium was amended with magnesium ammonium phosphate (7-40-6), iron sulfate, and a wetting agent (AquaGro CO., Cherry Hill, NJ). We stored pots in a cold room at 4°C for a period of 10 to 14 days to

break seed dormancy. Thereafter, pots were transferred into a greenhouse where seeds were allowed to germinate before the treatments started. Plantlets were thinned to one plant in each pot. Plants were fertilized with a 20-10-20 solution of "Peat-Lite Special" fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, OH) throughout the experiment. This solution was applied very often.

Radiation Environment

Plants under treatment received 11 kJ m^{-2} d^{-1} of biologically effective UV-B radiation, using fluorescent sunlamps (Q Panel UV-B 313, Cleveland, OH). Lamps over the treated plants were covered with a clear cellulose acetate film (0.13 mm thick) that absorbed shortwave UV-B and UV-C radiation but transmitted UV-B radiation wavelengths in the sunlight range. For the control treatment, identical lamps were used that were covered with polyester (Mylar) film (0.13 mm thick) that absorbed all UV-B radiation. We measured the UV flux with a double-monochromator spectroradiometer with holographic gratings (Optronic Model 742, Orlando, FL), calibrated against a 1000-W tungsten-halogen standard lamp traceable to the National Institute of Standards and Technology. Wavelength accuracy was checked before this calibration and again when the instrument was moved to the greenhouse by scanning a low-pressure mercury lamp with a discharge at distinct, well-separated emission lines. Biologically effective UV-B radiation was calculated by weighting the

spectral irradiance with a generalized plant action spectrum normalized to 300 nm (Caldwell 1971). This UV-B flux rate corresponded to UV-B on 21 June at 50° N at sea level with a 40% ozone reduction from normal levels. We calculated flux rates using the program "UV-B" (Fiscus and Booker ver. 3.02), which is based on the model of Björn and Murphy (1985). Supplementary visible radiation (200 $\mu mol.m^{-2}.s^{-1}$ for 14 h) was provided by several high-pressure sodium lamps. A quantum sensor (LI-COR, Lincoln, NE) was used to measure photosynthetically active radiation. Air temperature in the greenhouse was maintained at about 24°C.

Measured Traits

Plants were harvested before the onset of the seed pod drying stage. Morphological characters measured included plant height, rosette diameter, number of shoots and branches, and reproductive and vegetative dry mass. Leaf chlorophyll content of five of the parental ecotypes was measured using a nondestructive, dual-wavelength commercial meter (model SPAD-502, Minolta Corp., Ramsey, NJ; Monje and Bugbee 1992).

Experimental Design and Analysis of Data

The experiment was conducted in the spring of 1994 on two pairs of adjacent benches in a greenhouse; one bench of each pair was given supplemental UV-B radiation. Each pair of benches was considered a block, and both blocks contained all parental genotypes and hybrids. These blocks

were considered as two true replications. Five plants of each genotype (parents and their F1 hybrids) in each treatment were randomly distributed in a randomized complete block design on the benches.

Analysis of variance. Each response variable was analyzed separately based on means of the five subsamples in a three-way analysis of variance with treatment (T) and genotype (G) as fixed effects and block (B) as random effect. Interaction effects (T X B, T X G, and B X G) were also considered random effects. Since T and G were considered as fixed effects, it would have been more appropriate to consider the T X G interaction as a fixed effect. However, considering T X G interaction as a random effect results in more conservative tests for G and T main effects and since the interactions are tested over the error MS in either case, it does not affect the tests for any of the interactions (Fry JD, personal communication). Data were analyzed using the GLM procedure in SAS Release 6.12 (SAS, 1989) with the "test" option of the "Random" statement used to provide Satterthwaite approximate significance tests for the main effects. See Appendix B (Tables B.60-B.65) for a complete list of ANOVA tables.

Diallel analysis. Because the parental ecotypes used in our study were not collected from an individual breeding population, diallel analysis was performed based on model 1 and method 2 (parents and the F1 hybrids included), introduced by Griffing (1956). For this purpose, we conducted a general least squares analysis (Schaffer and Usanis 1995). For all response

variables, computation was based on the difference in values between plants given the elevated UV-B and those in the control group within each block (control - UV-B treated); a negative value indicated a better performance by the UV-B treated plants. Values for GCA and SCA were computed as suggested by Burow and Coors (1994) and Griffing (1956); these calculations involved the parental values. To compare individual GCA and SCA values, ecotype CS6079 and F1 hybrid CS6079 X CS6095 were taken as the standards, respectively. See Appendix B (Tables B.66-B.71) for a complete list of F-tests tables.

Results

Parental and F1 cross means for all traits represent the difference between mean response of control and UV-B-treated plants (Table 5). Significant genotype effects were detected for all traits, and there were significant treatment effects for plant height, rosette diameter, and vegetative and reproductive mass (Figure 4a, 4c). Number of shoots and branches increased for the treated plants, but these changes were marginally significant for shoots (P = .08) and not significant for branches (Figure 4b). The P-values for genotype, treatment, and the genotype X treatment interaction are presented in Table 6. Significant genotype X treatment interactions were found for plant height, rosette diameter, vegetative mass, and reproductive mass (P = .06).

Five of the parents were examined for chlorophyll content. On a leaf

area basis, chlorophyll concentration increased in the treated plants, but on a leaf mass basis, no treatment differences were observed (data not shown). Thus, the greater chlorophyll concentration on a leaf area basis under UV treatment was most likely due to a change in the specific leaf mass.

The GCA and SCA values for plant traits are based on the differences between control and treatment (control - UV-B-treated), the more negative numbers (meaning that the treated individuals, for example, grew taller or had higher dry mass than control plants) indicating a greater breeding value for parents and a superior phenotype for the hybrids (Table 7). Significant GCA values ($P < .06$) were detected for plant height and vegetative mass; SCA values were significant ($P < .05$) for rosette diameter and vegetative mass (Table 8).

Discussion

Our study is the first to use a diallel mating design to examine the inheritance of UV-B tolerance in plants. Analysis of diallel experiments provides information about heterosis and combining abilities to select superior crosses and parents. The relative contribution of additive and nonadditive gene action to inheritance of a trait can also be determined.

Significant general combining ability values were detected only for plant height and vegetative mass. For plant height, the mean squares of GCA were more than four times larger than that for specific combining ability, indicating a

much greater influence of additive effects than nonadditive gene effects for inheritance of UV-B tolerance in *Arabidopsis*. Desirable negative GCA values were found for plant height in CS6095, CS6094, CS6049, and CS6046, which makes them good general combiners for UV tolerance (Table 7). Negative and significant GCA values were also apparent for vegetative mass in CS6095 and CS6046. For vegetative mass, the mean squares of GCA and SCA were very similar, indicating approximately equal importance of additive and nonadditive gene effects for UV-B tolerance of this trait.

Significant SCA values and large heterotic effects suggest that hybrid production is a viable option to develop superior genotypes (Tables 7 and 9). Significant SCA values were obtained for rosette diameter and vegetative mass (Table 8). Hybrids CS6079 X CS6049, CS6094 X CS6049, and CS6094 X CS6047 produced the best SCA values for rosette diameter. Because GCA values were not significant for rosette diameter, hybrid production for UV-B tolerance may be sought for this trait. Favorable SCA values were obtained for vegetative mass in CS6079 X CS6049, CS6079 X CS6102, and CS6046 X CS6047. In the absence of significant SCA values, the performance of single-cross progeny can be predicted on the basis of GCA. Crosses of the two parents with the highest GCA estimates will have the best performing progeny (Baker 1978). For example, CS6095, CS6094, CS6046, and CS6049 were the parental genotypes with the highest GCA values for height, and four of their six F1 hybrids were among the five hybrids with the best SCA values.

The UV-B treatment was equivalent to solar noon UV-B on 21 June at 50° N at sea level with a 40% ozone reduction from normal levels, and no UV-B was provided in the control treatment. Because the geographical origins of these ecotypes were similar and the ecotypes were not selected for UV-B tolerance, this provided the opportunity to observe intrinsic variation in UV-B tolerance in these populations unrelated to the UV-B levels at their origin. This inherent variation in UV-B tolerance may have been in part the result of selection for genes encoding various defense mechanisms such as antioxidant enzymes or radiation shielding compounds, such as phenolics. While UV-B radiation might have been one selective environmental factor, other stresses such as pathogens, temperature extremes, or visible radiation may have been responsible for selection of such genes.

Because there are multiple traits with different values of GCA and SCA effects for each trait, choosing the most suitable genotypes or hybrids is dependent on the trait in question. One might select a particularly important trait that contributes to fitness (such as reproductive mass) or use a weighting formula that represents the relative importance of all desirable traits contributing to competitiveness and fitness. If all traits were treated equally, ecotypes CS6046 and CS6095 had the highest values for GCA, and CS6079 and CS6102 the lowest.

The role of genetic variation in responding to selective agents in the environment has been emphasized by many authors (e.g., Fisher 1930). Thus,

variation in UV-B tolerance among the different ecotypes of *Arabidopsis* and the control of at least some tolerance traits by additive gene effects should contribute to survival of this species in natural populations exposed to substantially elevated UV-B radiation. If similar variation exists for UV-B tolerance in agricultural crops, breeding for improved UV-B tolerance should be effective. Various levels of UV-absorbing compounds in different ecotypes are among the factors that may contribute to the observed ecotypic variation in UV-B tolerance (Hidema et al. 1996; Sato and Kumagai 1997).

The diallel mating system was used to establish the relative importance of both additive and non-additive gene effects in inheritance of UV-B tolerance in *Arabidopsis*. Significant GCA effects were present for plant height and vegetative mass. While the UV-B tolerance for plant height reduction in *Arabidopsis* is controlled more by additive than nonadditive gene effects, UV-B tolerance of reduced vegetative mass is influenced by both additive and non-additive gene effect. Significant SCA values were found for rosette diameter and vegetative mass. Our results suggest that selection for UV-B tolerance in *Arabidopsis* is possible given the amount of additive genetic effect among the parental ecotypes. The presence of significant SCA effects for some traits also suggests that hybrids can be used to develop UV-B tolerance. If these results for *Arabidopsis thaliana* are applicable to crop species, plant breeders would probably be able to develop pure lines and hybrids with improved tolerance to UV-B radiation.

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Table 4. *Arabidopsis thaliana* ecotypes and their origins

Ecotype	Origin	Latitude (N)
CS6046	Cologne, Germany	50° 56'
CS6047	Maidstone, Kent, UK	51° 16'
CS6049	Kent, UK	51° 12'
CS6079	Sidmouth, Devon, UK	50° 50'
CS6094	Brétigny-Sur-Org, France	48° 37'
CS6095	Brétigny-Sur-Org, France	48° 37'
CS6102	Kelsterbach, Germany	50° 03'

Table 5. Parental (in bold) and F1 mean values for height (H), number of shoots (S), number of branches (B), rosette diameter (D), vegetative mass (V), and reproductive mass (R)

	Character	CS6079	CS6095	CS6094	CS6046	CS6049	CS6047	CS6102
CS6079	H	**7.72**	6.73	14.33	10.05	8.00	7.28	10.01
	S	**0.00**	2.20	-0.25	0.20	0.10	-0.10	0.00
	B	**0.20**	0.30	0.00	-0.20	-0.20	0.40	0.40
	D	**0.23**	0.06	3.15	-0.35	-0.04	0.11	0.02
	V	**0.25**	0.05	0.70	0.17	-0.01	0.31	0.00
	R	**0.25**	0.04	0.26	0.18	-0.02	0.30	- 0.01
CS6095	H		**2.96**	4.96	-1.03	-3.55	1.53	0.00
	S		**-0.10**	-0.72	-0.70	-0.10	0.20	0.00
	B		**0.90**	-0.39	-3.00	-1.60	-1.10	0.00
	D		**0.75**	1.83	-0.17	0.23	0.72	0.37
	V		**0.08**	-0.02	0.13	0.13	0.41	0.21
	R		**0.01**	-0.14	-0.01	-0.03	0.01	0.00
CS6094	H			**9.39**	3.35	0.55	10.15	9.26
	S			**0.40**	-1.4	-2.13	-2.50	-0.30
	B			**0.10**	0.30	-0.28	0.10	0.00
	D			**-0.24**	0.86	0.40	-0.34	1.88
	V			**0.11**	0.12	0.06	0.21	0.34
	R			**0.09**	-0.03	0.11	0.21	0.03
CS6046	H				**4.52**	9.36	5.07	7.56
	S				**-2.50**	1.90	0.10	0.40
	B				**-0.50**	0.10	-0.20	0.10
	D				**-0.17**	0.14	0.45	0.67
	V				**-0.18**	0.33	-0.26	0.37
	R				**-0.18**	0.31	-0.28	0.35

Table 5. Continued.

	Character	CS6079	CS6095	CS6094	CS6046	CS6049	CS6047	CS6102
CS6049	H					**6.40**	8.09	9.93
	S					**0.10**	-2.42	0.17
	B					**0.30**	-0.25	-0.17
	D					**1.36**	0.46	0.95
	V					**0.20**	0.23	0.60
	R					**0.15**	0.21	0.58
CS6047	H						**4.56**	10.82
	S						**-0.30**	-0.80
	B						**0.40**	-0.10
	D						**1.10**	0.70
	V						**0.05**	0.26
	R						**-0.01**	0.26
CS6102	H							**10.83**
	S							**-2.90**
	B							**0.10**
	D							**-0.39**
	V							**0.33**
	R							**0.34**

Table 6. Probability values for genotype, treatment, and the interaction for genotype and treatment (G x T)

Character	Genotype	Treatment	G x T
Height	.0001	.0001	.0020
Number of shoots	.0001	.0838	.4946
Number of branches	.0003	.4315	.9518
Rosette diameter	.0001	.0001	.0247
Vegetative mass	.0001	.0001	.0252
Reproductive mass	.0001	.0004	.0620

Table 7. General combining ability (in bold) and specific combining ability for plant height (H), number of shoots (S), number of branches (B), rosette diameter (D), vegetative mass (V), and reproductive mass (R). For all response variables, computation was based on the difference in values between plants given the elevated UV-B and those in the control group within each block (control - UV-B treated)

	Character	CS6079	CS6095	CS6094	CS6046	CS6049	CS6047	CS6102
CS6079	H	**2.31**	2.10	4.49	2.21	-0.04	-1.52	-0.70
	S	**0.60**	1.57	-0.09	0.14	-0.20	0.02	0.15
	B	**0.26**	0.50	-0.23	-0.01	-0.24	0.20	0.11
	D	**-0.11**	-0.48	2.39[d]	-0.43	-0.52	-0.32	-0.44
	V	**0.03**	-0.12	0.47[b]	0.07	-0.25	0.12[a]	-0.31
	R	**0.04**	0.00	0.13	0.10	-0.24[a]	0.17	- 0.28[a]
CS6095	H		**-4.06**[c]	1.49	-2.50[a]	-5.22[c]	-0.91	-4.34[b]
	S		**0.44**	-0.39	-0.59[a]	-0.24	0.49	0.31
	R		**-0.31**	-0.06	-2.24[b]	-1.08	-0.74	0.28
	D		**0.10**	0.86[b]	-0.46	-0.47	0.09	0.46[a]
	V		**-0.05**	-0.18	0.10	-0.04	0.30[b]	-0.03
	R		**-0.11**[b]	-0.11	0.07	-0.09	0.03	-0.11
CS6094	H			**-1.14**[b]	-3.32[b]	-6.32[d]	2.51	-0.29
	S			**-0.36**[a]	-0.50[a]	-1.47[b]	-1.42[b]	0.81
	B			**0.13**	0.62	-0.19	0.03	-0.16
	D			**0.32**[a]	0.34[a]	-0.52	-1.19	0.99[b]
	V			**0.02**	0.03	-0.17	0.03	0.03
	R			**-0.02**	-0.03	-0.04	0.14	-0.17
CS6046	H				**-0.86**[b]	4.49	-0.57	0.01
	S				**-0.14**	2.34	0.96	1.29
	B				**-0.30**	0.61	0.15	0.37
	D				**-0.35**	-0.11	0.26	0.45[a]
	V				**-0.11**[b]	0.23[b]	-0.31	0.19[b]
	R				**-0.08**[a]	0.21	-0.29[a]	0.20

Table 7. Continued

Character		CS6079	CS6095	CS6094	CS6046	CS6049	CS6047	CS6102
CS6049	H						**-0.66[b]**	2.25	2.19
	S						**0.11**	-1.80[b]	0.80
	B						**-0.06**	-0.13	-0.13
	D						**0.05**	-0.13	0.33
	V						**0.03**	0.04	0.28[b]
	R						**0.07**	0.05	0.29[a]
CS6047	H							**0.11[b]**	2.31
	S							**-0.32[a]**	0.27
	B							**0.10**	-0.23
	D							**-0.01**	0.14
	V							**-0.02**	-0.01
	R							**-0.02**	0.06
CS6102	H								**2.02**
	S								**-0.34[a]**
	B								**0.18**
	D								**0.02**
	V								**0.11**
	R								**0.12**

[a], [b], [c], and [d] indicate estimates are significant at $P < .1$, .05, .01, and .005, respectively.

Table 8. Mean squares and *P*-values for general and specific combining abilities (GCA and SCA, respectively)

Character	GCA	SCA	Error
Height	85.01[c]	20.97	13.67
Number of shoots	2.79	2.95	2.4
Number of branches	0.94	1.11	1.68
Rosette diameter	0.73	1.42[b]	0.57
Vegetative mass	0.82[a]	0.84[a]	0.41
Reproductive mass	0.12	0.56	0.40

[a], [b], and [c] indicate estimates are significant at $P < .1$, .05, and .001, respectively.

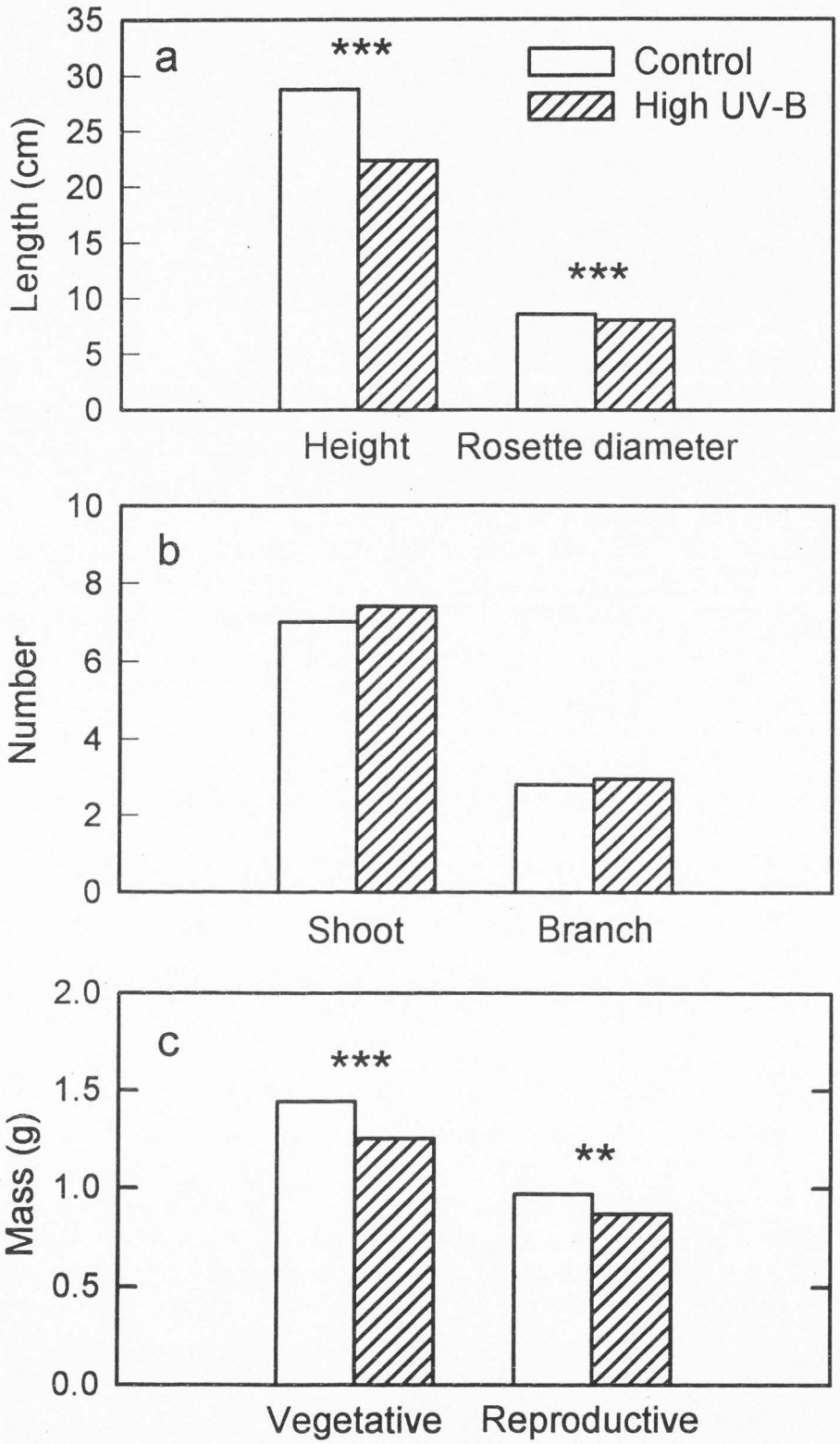
Table 9. Heterosis by cross, relative to midparent (cross - midparent) for plant height (H), number of shoots (S), number of branches (B), rosette diameter (D), vegetative mass (V), and reproductive mass (R). Units of measure are expressed as centimeter and gram for height and rosette diameter, and vegetative and reproductive mass, respectively. A negative number translates to a better performance by the cross

	Character	CS6095	CS6094	CS6046	CS6049	CS6047	CS6102
CS6079	H	1.39	5.77	3.93	0.94	1.14	0.74
	S	2.25	-0.45	1.45	0.05	0.05	1.45
	B	-0.25	-0.15	-0.05	-0.45	0.10	0.25
	D	-0.44	3.15	-0.38	-0.83	-0.56	0.10
	V	-0.12	0.51	0.14	-0.23	0.16	- 0.28
	R	-0.09	0.09	0.14	-0.22	0.18	- 0.31
CS6095	H		-1.22	-4.77	-8.23	-2.23	-6.90
	S		-0.87	0.60	-0.10	0.40	1.50
	B		-0.89	-3.20	-2.20	-1.75	-0.50
	D		1.57	-0.46	-0.83	-0.21	0.94
	V		-0.12	0.18	-0.02	0.34	0.01
	R		-0.19	0.08	-0.10	0.01	-0.17
CS6094	H			-3.61	-7.35	3.18	-0.85
	S			-0.35	-2.38	-2.55	0.95
	B			0.50	-0.48	-0.15	-0.10
	D			1.06	-0.16	-0.77	2.19
	V			0.16	-0.10	0.13	0.12
	R			0.02	-0.01	0.17	-0.19
CS6046	H				3.90	0.53	-0.12
	S				3.10	1.50	3.10
	B				0.20	-0.15	0.30
	D				-0.46	-0.02	0.95
	V				0.32	-0.19	0.30
	R				0.32	-0.18	0.26

Table 9. Continued

	Character	CS6095	CS6094	CS6045	CS6049	CS6047	CS6102
CS6049	H					2.61	1.32
	S					-2.32	1.57
	B					-0.60	-0.37
	D					-0.77	0.47
	V					0.11	0.34
	R					0.13	0.33
CS6047	H						3.13
	S						0.80
	B						-0.35
	D						0.34
	V						0.07
	R						0.10

Figure 4. The effect of UV-B radiation on morphological traits and reproductive and vegetative dry mass of *Arabidopsis thaliana*: (a) Plant height (cm) and rosette diameter (cm); (b) vegetative and reproductive dry mass (g); (c) number of shoots and branches on the main shoot. Treatment effect was significant at $P < .001$ for plant height, rosette diameter, and reproductive and vegetative dry mass. This effect was significant at $P < .1$ for shoot number.


a
***
Control
High UV-B
***
Length (cm)
35
30
25
20
15
10
5
0
Height
Rosette diameter
b
Number
10
8
6
4
2
0
Shoot
Branch
c
***
**
Mass (g)
2.0
1.5
1.0
0.5
0.0
Vegetative
Reproductive

CHAPTER 4

MORPHOLOGICAL AND FLOWERING TIME RESPONSES OF *ARABIDOPSIS THALIANA* IN A MULTIPLE GENERATION EXPOSURE TO ULTRAVIOLET-B RADIATION[1]

Abstract

Response due to exposure to ultraviolet-B (UV-B) radiation through several generations may accumulate and be expressed in later generations, even in the absence of UV-B. The objectives of our study were to (1) determine if such a cumulative effect of UV-B exposure through multiple generations occurs in *Arabidopsis thaliana,* and (2) evaluate if this cumulative effect is more pronounced in the seventh than third generation. We grew three ecotypes of *Arabidopsis* for seven generations under either the presence or absence of UV-B. Seeds obtained from the third and seventh generations were germinated and used for a final generation (final experiment) in which one set of each treatment group (exposure history) received a high level of UV-B, and the other set received no UV-B. This final generation was used to test for possible cumulative effects. Several morphological traits were measured including plant height, number of shoots and branches, rosette diameter, and vegetative and

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[1]Coauthored by Javad Torabinejad, Martyn M. Caldwell, Susan Durham, and Paul Wolf. See Appendix A of this dissertation for the release letters of the two coauthors who are not on the supervisory committee.

reproductive dry mass. We also examined leaf chlorophyll content and UV-pigment extract absorbance at several wavelengths. A significant carryover effect of exposure history (H) was observed only for days to flowering. However, the ecotypes reacted very differently to UV-B and often with contrasting results, which likely obscured a significant H main effect. However, there were significant H X ecotype interactions, and several carryover effects for H were manifested in both the absence and presence of UV-B in the final experiment for the individual ecotypes. The H effects were more pronounced in the third than seventh generation. Thus, rather than a continued accumulation of radiation exposure carryover effect through the generations of *Arabidopsis*, there was an apparent reversion of this H effect, implying that both positive and negative effects of UV-B exposure history in earlier generations fade away in later generations.

Introduction

Experiments involving a single generation of plants, usually in a single growing season, have demonstrated a variety of morphological, physiological, and molecular responses to ultraviolet-B (UV-B) radiation (Strid *et al.* 1994; Björn 1996; Britt 1996; Greenberg *et al.* 1997; McLeod & Newsham 1997; Rozema *et al.* 1997; Jansen *et al.* 1998; Vonarx *et al.* 1998; Caldwell *et al.* 1998). Much less, however, is known about the cumulative effects of UV-B radiation exposure in subsequent growing seasons for perennial plants or

subsequent generations for annual plants.

In a 3-year study, Sullivan and Teramura (1992) reported an apparent accumulation of UV-B effects for *Pinus taeda* (loblolly pine). Although there were no effects in the first season, by the end of the third year, biomass was reduced by UV-B relative to controls in at least one ecotype. Johanson *et al.* (1995) reported a similar finding for an evergreen subarctic shrub, *Empetrum hermaphroditum*. This apparent accumulation of UV-B effects was not evident in the other three shrub species studied.

Accumulated carryover effects of elevated UV-B were investigated in a series of studies with *Dimorphotheca sinuata*, an annual species in the Asteraceae (Musil, 1996; Midgley *et al.* 1998; Musil *et al.* 1999). In the first study (Musil, 1996), the same seed source was used for populations of *D. sinuata* that were grown under two levels of UV-B radiation for two consecutive generations in a greenhouse. The third generation of each treatment group was cultivated outdoors under two UV-B radiation levels. Plant performance was influenced more by UV-B exposure history than by UV-B exposure in the third generation. The accumulative carryover effect of UV-B in the first two generations resulted in significant reductions in several parameters including leaf area and number of stems, reproductive dry mass, and pollen tube growth rates, but induced earlier reproductive activity. These carryover cumulative effects were attributed to altered DNA integrity.

In another study, a fifth generation of *D. sinuata* plants was grown in a

UV-B free greenhouse to test for accumulated effects of UV-B exposure in the previous generations (Midgley *et al.* 1998). At the time of flowering, leaves were collected and assessed for leaf fluctuating asymmetry, a measure of developmental instability. The asymmetry increased due to UV-B exposure in previous generations. However, other leaf morphological traits did not show a significant response to UV-B exposure history. In a similar study with the same species previously exposed to four generations of UV-B exposure, Musil *et al.* (1999) tested carryover effect of four generations grown under UV-B for the fifth generation in the absence of UV-B. They found several characteristics were affected by previous UV-B exposure including reduced photosynthetic rates, leaf polyphenolics, apical dominance, and seed production; however, there was greater branching and reproductive structures and earlier flowering.

In a preliminary experiment, we observed differential responses among four ecotypes of *Arabidopsis thaliana* exposed to UV-B in the course of four generations. In the fifth generation plants grown both with and without UV-B had a greater height for the treatment group with a UV-B exposure history for one ecotype, but the other ecotypes exhibited no such response or were shorter. It is suggested that carryover effects of UV-B irradiation in subsequent generations may amplify changes caused by UV-B effects (Caldwell *et al.* 1998).

The present study was designed to (1) test for carryover effects of UV-B exposure in previous generations in three ecotypes of *Arabidopsis*, and (2)

determine if plants exposed to UV-B for seven generations would exhibit more pronounced cumulative effects of exposure history than plants exposed to three generations.

Materials and methods

Overview

To assess the carryover effects of UV-B exposure history (H), we grew three ecotypes of *Arabidopsis thaliana* for seven generations under presence or absence of UV-B radiation. These ecotypes came from various locations in Europe (Table 10); the seed was obtained from the *Arabidopsis* Biological Resource Center at Ohio State University. In an earlier trial mentioned above, these ecotypes differed in their sensitivity to UV-B radiation. From December 1993 to October 1995, plants were grown for seven consecutive generations in a greenhouse; one group received a high level of UV-B and the other no UV-B. Seeds of plants from the two exposure histories were germinated and used for the final experiment in which they were tested for H carryover effects under both the presence and absence of UV-B. To determine if there was a progressive accumulative of effect due to H through the generations, we also included plants with three generations of exposure history in the final experiment (Fig. 5). In this manner the effect of three and seven generations of H could be compared.

Growth conditions and radiation exposure

Several seeds of each ecotype were sown separately in a medium consisting of equal proportions of vermiculite, bark, peat moss, and perlite. This potting medium was amended with magnesium ammonium phosphate (7-40-6), iron sulfate, and a wetting agent (AquaGro Co., Cherry Hill, NJ). We stored pots in a cold room at 4 °C for a period of 10-14 days to break seed dormancy. Thereafter, pots were transferred into a greenhouse where seeds were allowed to germinate before treatments started. Seedlings were thinned to one per pot. Plants were fertilized with a 20-10-20 solution of "Peat-Lite Special" fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, OH) throughout the experiment. This solution was applied very often.

During the multigeneration radiation exposures, one treatment group consistently received a high level of UV-B (9-12 kJ m^{-2} d^{-1} of biologically effective UV-B, UV-B_{BE}) radiation and the other received no UV-B. For the UV-B treatment, fluorescent sunlamps (Q Panel UV-B 313, Cleveland, OH) were erected over the plant bed. Lamps above the UV-B-treated plants were covered with a clear cellulose acetate film (0.13 mm thick), which absorbed shortwave UV-B and UV-C radiation but transmitted UV-B radiation wavelengths in the sunlight range (ca. > 290 nm). For the control, identical lamps were covered with polyester (Mylar) film (0.13 mm thick) that absorbs all UV-B and UV-C radiation. We measured the UV flux with a double-monochromator spectroradiometer with holographic gratings (Optronic Model

742, Orlando, FL), calibrated against a 1000-W tungsten-halogen standard lamp traceable to the National Institute of Standards and Technology. Wavelength accuracy was checked before this calibration and again when the instrument was moved to the greenhouse by scanning a low-pressure mercury lamp with a discharge at distinct, well-separated, known emission lines. Biologically effective UV-B radiation was calculated by weighting the spectral irradiance with a generalized plant action spectrum (Caldwell 1971) normalized to 300 nm. The highest UV-B flux employed (12 kJ m^{-2} d^{-1} $UV\text{-}B_{BE}$) corresponded to daily solar UV-B on 21 June at 50° N at sea level with a 43% ozone reduction from normal levels. We calculated flux rates using the program "UV-B" (Fiscus and Booker ver. 3.02), which is based on the model of Björn and Murphy (1985). We also provided supplementary visible radiation (90 μmol m^{-2} s^{-1} for 8-15 h d^{-1}) from either high pressure sodium or metal halide lamps. A quantum sensor (LI-COR, Lincoln, NE) was used to measure photosynthetically active radiation. Air temperature in the greenhouse was maintained at 24 °C. Plants in the final test experiment received 12 kJ m^{-2} d^{-1} $UV\text{-}B_{BE}$. Supplementary visible radiation (430 μmol m^{-2} s^{-1} for 6 h d^{-1}) was provided by several high-pressure sodium lamps.

Plant measurements

At the conclusion of the final experiment conducted in the summer of 1997, plants were harvested before onset of the seed pod drying stage. In

addition to reproductive and vegetative dry mass, several morphological characters were determined including plant height, rosette diameter, and number of shoots and branches. Leaf chlorophyll content was measured using a nondestructive, dual-wavelength commercial meter (model SPAD-502, Minolta Corp., Ramsey, NJ; Monje & Bugbee 1992) and expressed on both a leaf mass and leaf area basis. To assess the crude amounts of anthocyanins and other phenolic compounds, we followed the method of Beggs and Wellmann (1985). Three leaf disks (1 cm^{-2}) were placed in a vial containing 3 ml of methanol:HCl solution (99:1, v:v); the solution was kept in the dark at 20°C until used. A spectrophotometer (Beckman DU-7, Beckman Instruments, Inc., Irvine, CA) was used to measure absorbance at 260, 305, 320, 355, 398, 531, and 654 nm. Absorbance at 531 nm was used for anthocyanin measurements. During the experiment, the time of flowering was observed.

Experimental design

The final experiment tested for the influence of UV-B exposure history treatment (H; 2 levels: presence or absence of UV-B), generations (G; 2 levels: plants from third and seventh generations), UV-B treatment in the final experiment (P; 2 levels: presence or absence of UV-B), and ecotype (E; 3 levels). Forty-eight pots were arranged on six tables in the greenhouse; each table was randomly assigned to one level of P, and each pot represented one of 12 treatment combinations defined by factors H, E, and G. There were four

replicates of each treatment combination on each table. To reduce variability among tables, the tables were rotated in the greenhouse every 3 days.

Analysis of variance of a four-way factorial in a split-plot design was performed to assess the differences among ecotype and effect of UV-B exposure during previous generations (H) and in the final experiment for morphological traits, vegetative and reproductive mass, chlorophyll and anthocyanin content, and pigment absorbance at several wavelengths. We used means of four replicate treatment combinations on each table for the analysis. Computations were done using PROC MIXED in SAS (Release 6.12). See Appendix B (Tables B.72-B.89) for a complete list of ANOVA tables.

Results

The test of UV-B exposure over several generations was evaluated in the final experiment, and there were indications of carryover effects of UV-B exposure in previous generations. The relative response of the three ecotypes to UV-B exposure history (H) was tested in both the presence and absence of UV-B in the final experiment, and the patterns were virtually identical. Therefore, the average of these tests is shown in Figure 6. Table 11 provides *P*-values for effects of ecotype (E), generation (G), exposure history (H), and presence or absence of UV-B (P) in the final treatment, and their various interactions. Except for days to flowering, there was not a significant effect of previous UV-B exposure (H) for any of the plant characters. However, this

appears to be primarily due to the different directions and magnitudes of response to UV-B exposure history by the different ecotypes (Fig. 6); there are significant ecotype X H interactions for several traits (Table 11). For example, due to the UV-B exposure history, the number of shoots, UV-absorbing pigments, and chlorophyll increased in one ecotype, decreased in another and exhibited little response in a third ecotype (Fig. 6a). Specific leaf mass increased in two ecotypes, but not in the other (data not shown). Thus, exposure to UV-B through several generations was manifested in the final generation, but the ecotypes responded very differently.

Plants from the third and seventh generations were compared for their relative response to UV-B exposure history because we wanted to determine if the cumulative influence of UV-B increased as subsequent generations were exposed to UV-B. Differences were found for several traits between the third and seventh generations (Fig. 6b), and this is evident in the significant E X G X H interactions for many traits (Table 11). Unexpectedly, the third generation exhibited generally much more response to the UV exposure history than the seventh (Fig. 6b).

Not surprisingly, there were several significant ecotypic differences and significant responses to UV-B in the final experiment (overall significant E and P main effects for several traits).

Discussion

There were clearly some carryover effects of UV-B exposure in earlier generations that were evident in the final test experiment, and these were apparent both in the presence and absence of UV-B in the final experiment. The demonstration of carryover effects generally corresponds with the findings of Musil and colleagues with the desert annual, *Dimorphotheca sinuata* (Musil 1996; Midgley *et al.* 1998; Musil *et al.* 1999). Furthermore, we tested the degree to which the carryover effects might accumulate as subsequent generations were exposed to UV-B radiation in the comparison of the third and seventh generations. Contrary to our expectations, instead of being more pronounced in the seventh generation, the effects of UV exposure history were considerably dampened relative to the third generation. Thus, there was an apparent reversion of cumulative radiation effect. An increasing accumulation of UV-B exposure effect in consecutive years has been suggested to occur in two perennial evergreen species (Sullivan & Teramura 1992; Johanson *et al.* 1995), but the evidence for this is not strong and limited to a couple of years of treatment. A tendency for accumulation of UV-B exposure effect on leaf fluctuating asymmetry in the annual *Dimorphotheca sinuata* when multiple generations were exposed to UV-B was reported by Midgley *et al.* (1998). This tendency in *Dimorphotheca* is in contrast to our findings in the comparison of the third and seventh generation of *Arabidopsis*.

As explained at the outset, many of the traits that were exhibited as

carryover effects of the earlier exposure in the studies of the Musil group can be interpreted as symptoms of damage, e.g., reductions in dry mass (leaves, reproductive organs, and stems), stem number, inflorescence production, and pollen tube growth rates (Musil 1996). Only one population of this annual was used in these experiments. In our experiments, we used three ecotypes from middle and northern Europe where the solar UV climate most likely does not differ greatly among the sites of origin. The manifestations of the UV-B exposure history differed decidedly among the ecotypes, both in degree and direction, as depicted earlier. Many of the effects of UV-B may be interpreted as photomorphogenetic in nature and mediated by a putative UV-B receptor (Ballaré *et al.* 1991, 1992, 1995; Caldwell & Flint 1994; Caldwell *et al.* 1998). If the responses evident in our experiments are more photomorphogenetic in nature rather than expressions of damage, it is less surprising that these may appear in different directions among the ecotypes. However, the explanation for these rather striking differences is wanting.

Musil (1996), Midgley *et al.* (1998), and Musil *et al.* (1999) have attributed the long-term effects of UV-B to mutations that can accumulate and carry over into subsequent generations. DNA damage can occur even under normal solar radiation; however, mutations generated by UV-B irradiation are rare or nonexistent and have not been isolated in *Arabidopsis* (Murphy, TM and Britt, AB, personal communication).

In conclusion, we found that: (1) UV-B exposure history effects can be

identified in *Arabidopsis;* (2) there is an apparent reversion in the UV exposure history influence, possibly indicating a lessened role over several generations; and (3) H effects are highly ecotype specific. Our study suggests that both positive and negative effects of exposure history in earlier generations fade away in later generations. It remains to be explored whether this reversion in the UV-B exposure history influence can cause differences in plant performance in each generation. We did not calculate relative differences among plants under control and treatment for individual generations.

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Table 10. *Arabidopsis thaliana* ecotypes and their origins.

Ecotype	Origin	Latitude (N)
CS6049	Kent, UK	51° 12'
CS6079	Sidmouth, Devon, UK	50° 50'
CS6094	Brétigny-Sur-Org, France	48° 37'

Table 11. *P*-values ($p < 0.05$) for treatment in the final experiment (T), ecotype (E), generation (G), and exposure history (H) effects and their interactions.

Character	T	E	G	H	TXE	TXG	EXG	TXH	EXH	GXH	TXEXG	TXEXH	TXGXH	EXGXH	TXEXGXH
Height	0.0003	0.0018	0.0033	ns	ns	ns	0.0004	ns	ns	ns	ns	ns	0.0430	ns	0.0007
Shoot number	ns	0.0001	ns	ns	ns	ns	0.0001	ns	0.0001	ns	ns	ns	ns	0.0001	ns
Branch number	ns	0.0004	ns	ns	ns	ns	0.0009	ns	0.0506	ns	ns	0.0136	ns	ns	ns
Rosette diameter	ns	ns	0.0204	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.0131	ns
Vegetative mass	0.0260	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.0083	ns
Reproductive mass	0.0396	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.0120	ns
R mass / V mass	ns	0.0059	0.0001	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Specific leaf mass	ns	0.0067	ns	ns	ns	ns	0.0396	ns	0.0008	ns	ns	ns	ns	0.0025	ns
Days to flowering	ns	0.0001	0.0001	0.0438	0.0516	ns	0.0001	ns	0.0001	ns	ns	ns	ns	0.0001	ns
Chlorophyll mg m^{-2}	0.0008	0.0395	ns	ns	ns	ns	0.0061	ns	ns	ns	ns	ns	ns	ns	0.0454
Chlorophyll mg g^{-1}	ns	ns	ns	ns	ns	0.0432	ns	ns	0.0040	ns	ns	ns	ns	0.0009	ns
260 nm	ns	0.0001	ns	ns	ns	ns	0.0005	0.0315	0.0003	ns	ns	ns	ns	0.0001	ns
305 nm	ns	0.0001	ns	ns	ns	ns	0.0002	ns	0.0003	ns	ns	ns	ns	0.0010	ns
320 nm	0.0329	0.0001	ns	ns	ns	ns	0.0005	ns	0.0016	ns	ns	ns	ns	0.0022	ns
355 nm	0.0117	0.0001	ns	ns	ns	ns	0.0041	ns	0.0199	ns	ns	ns	ns	0.0101	ns
398 nm	ns	0.0006	ns	ns	ns	ns	0.0313	ns	0.0004	ns	ns	ns	ns	0.0001	ns
531 nm	ns	0.0001	ns	ns	ns	ns	0.0013	ns	0.0001	ns	ns	ns	ns	0.0001	ns
654 nm	ns	0.0114	ns	ns	ns	ns	ns	ns	0.0025	ns	ns	ns	ns	0.0001	ns

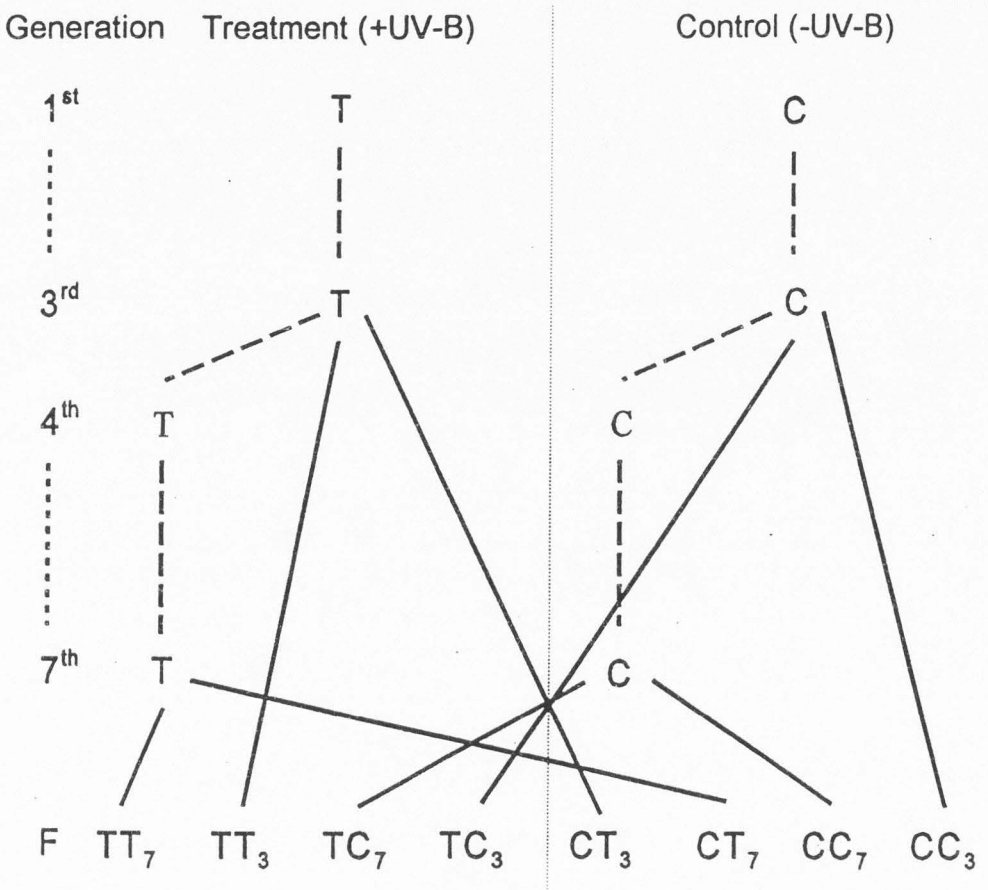
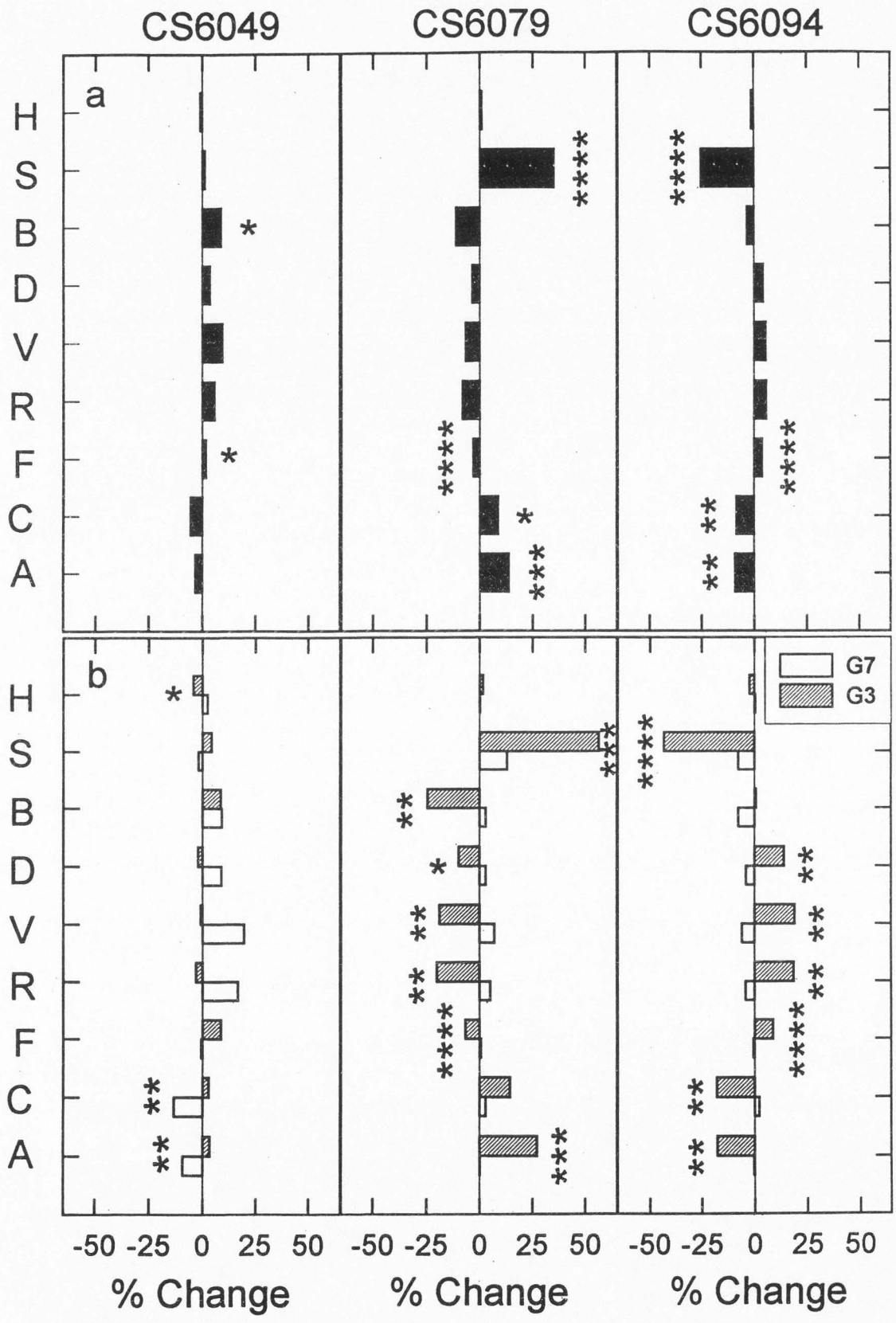


Fig. 5. Experimental design for testing the carryover effects of UV-B exposure through multiple generations in a final test in the presence and absence of UV-B. The design also incorporates a comparison of carryover effects from the third and seventh generations. Plants under UV-B treatment and control are designated as T and C, respectively, for both the exposure history and presence or absence of UV-B in the final test experiment. For the letter code designations in the final experiment (F), the first letter of the pair in each case refers to the presence or absence of UV-B in the final experiment and the second letter refers to UV-B treatment or control during the exposure history; the subscript designates the generation being tested for carryover effect.

Fig. 6. Percentage in plant traits due to the exposure history treatment (H) relative to the control in three ecotypes of *Arabidopsis thaliana* (a) for the overall H main effect and (b) for generations three and seven. The abbreviations H, S, B, D, V, R, F, C, and A designate plant height, number of shoots, number of branches on the main shoot, diameter of the rosette, vegetative mass, reproductive mass, days to flowering, chlorophyll content per leaf mass, and absorbance reading at 320 nm, respectively. *, **, ***, and **** refer to *P* values < 0.1, 0.05, 0.01, and < 0.001, respectively. The exposure history effect portrayed here is the average of plants tested in the presence and absence of UV-B in the final experiment. Very similar patterns were observed for plants tested in the presence and absence of UV-B in the final experiment, therefore, these are not shown separately.


CS6049
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CHAPTER 5

SUMMARY

Certain anthropogenic activities have resulted in a reduction in stratospheric ozone. Consequently, more ultraviolet-B (UV-B) radiation reaches the earth's surface, and this can have implications for biological systems, including higher plants. Despite recent international agreements to reduce or ban the release of ozone-depleting chemicals into the atmosphere, many uncertainties exist about the future of stratospheric ozone levels (Madronich *et al.* 1998). Although during the past nearly three decades extensive research has demonstrated various plant responses to UV-B radiation (Caldwell & Flint 1994), there are still many aspects of UV-B research that need to be addressed. These include UV-B effects on plant reproduction, inheritance of UV-B tolerance, and long-term effects of UV-B on plants.

In Chapter 2, I explored responses of pollen to UV-B *in vitro*. I demonstrated that UV-B can reduce pollen tube growth in many species, but only reduces pollen germination in a few species. My study was unique because it involved a large number of plant species (34 taxa) representing several families, under comparable conditions and UV-B flux rates, and also employed a higher level of visible radiation than used in other investigations. The magnitude of visible radiation is important because it allows pollen the opportunity for photorepair of some of the UV-B damage and corresponds more

closely with natural conditions. Because a reduction in pollen germination and pollen tube growth may result in reduced pollination success, this may have implications for maintaining competitive plant populations in nature. However, additional *in vivo* studies are needed in which pollen performance can be monitored under field conditions.

Considerable interspecific and intraspecific variation has been observed in plant response to UV-B radiation (Kossuth & Biggs 1981; Teramura 1983; Tevini & Teramura 1989; Barnes *et al.* 1990; Sullivan *et al.* 1992; Ziska *et al.* 1992; Day 1993; Corlett *et al.* 1997; McLeod & Newsham 1997; Correia *et al.* 1998; Torabinejad *et al.* 1998). Strong genotypic differences were observed for all of the traits examined in my study of *Arabidopsis* in Chapter 3. Furthermore, significant general and specific combining abilities existed for a number of characters. If my results for *Arabidopsis thaliana* are applicable to other species, plant breeders may develop either pure lines or hybrids of species that can be differentially responsive to elevated UV-B radiation. This suggests that plant populations with sufficient genetic diversity in responsiveness to UV-B radiation may be selected for improved response to UV-B.

Accumulative UV-B effects through multiple growing seasons have been suggested in investigations with a few perennial plant species (Sullivan & Teramura 1992; Johanson *et al.* 1995), although this evidence has not been particularly strong. However, a rather convincing case for carryover effects of previous UV-B exposure into subsequent generations of an annual species has

been presented (Musil 1996; Midgley *et al.* 1998; Musil *et al.* 1999). These data, however, only weakly support the contention that the effects of UV-B exposure in subsequent generations accumulate through the generations (Musil *et al.* 1999). Although ecotypes of *Arabidopsis thaliana* responded rather differently, I demonstrated in Chapter 4 that there is a carryover effect of UV-B exposure history in the third generation. However, this accumulative effect was not observed in the seventh generation. Instead, there was an unexpected reversion of the carryover effect in the seventh compared to the third generation. This is the first time that this phenomenon has been reported.

Overall, in these experiments I showed that UV-B radiation altered both reproductive and vegetative responses. I also showed that a range of tolerance to UV-B radiation existed in different species and different genotypes of the same species. If such variation in UV-B responsiveness occurs generally in plant populations, there is an apparent opportunity for selection.

The necessity to maintain strict control over UV-B radiation limited my work to growth chamber and greenhouse environments. Plants behave quite differently in these artificial environments compared to field conditions in terms of UV-B responsiveness, although the reasons for these discrepancies are not entirely known (Teramura 1983; Caldwell *et al.* 1995). Confirming the results of my work under field conditions should be a high priority.

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APPENDICES

APPENDIX A. LETTERS

American Journal of Botany
Section of Plant Biology, Cornell University,
Ithaca NY 14853 Phone 607-254-4708 Fax 607-254-4695

May 6, 1999

Mr. Javad Torabinejad
Rangeland Resources Department
Utah State University
Logan, UT 84322-5230

Dear Mr. Torabinejad:

This letter provides you with authorization from the *American Journal of Botany* to use any and all material necessary from:

Torabinejad, J., M. M. Caldwell, S. D. Flint, and S. Durham. 1998. Susceptibility of pollen to UV-B radiation: an assay of 34 taxa. *American Journal of Botany* 85(3): 360-369

in your Ph.D. dissertation to be submitted to Utah State University.

Sincerely,

Karl Niklas (Editor-in-Chief)

Utah State
UNIVERSITY

DEPARTMENT OF FISHERIES AND WILDLIFE
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May 6, 1999

Javad Torabinejad
Utah State University
Department of Rangeland Resources
5230 Old Main Hill
Logan, UT 84322-5230

Dear Javad:

You have my permission to include in your dissertation the papers on which I am co-author: *Susceptibility of pollen to UV-B radiation: an assay of 34 taxa* and *Morphological and flowering time responses of* Arabidopsis thaliana *in a multiple generation exposure to ultraviolet-B radiation.*

Sincerely,

Susan Durham

COLLEGE OF NATURAL RESOURCES

Utah State
UNIVERSITY

DEPARTMENT OF RANGELAND RESOURCES
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May 6, 1999

Dear Javad,

As a coauthor, I give my consent to the use of the paper "Susceptibility of pollen to UV-B radiation: An assay of 34 taxa", which was published in American Journal of Botany in 1998, as a chapter in your dissertation.

Sincerely,

Stephan Flint
Research Associate

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Wednesday, May 12, 1999

Javad Torabinejad
Department of Rangeland Resources
Utah State University
Logan, UT 84322

Dear Javad,

As a coauthor on the manuscript entitled "Morphological and flowering time responses of Arabidopsis thaliana in a multiple generation exposure to ultraviolet-B radiation", you have my permission to use the manuscript as part of you dissertation in fulfilment of your Ph.D. at Utah State University. Good luck

Paul G. Wolf
Associate Professor, Biology

Phone (801) 797-2485 Fax (801) 797-1575 biomail@cc.usu.edu

APPENDIX B. STATISTICAL TABLES

Table B.1. ANOVA for relative change in pollen tube length of *Allium ostrowskianum*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	159.33	159.33	8.72	0.2079
Trial	1	0.75	0.75	0.04	0.8724
UV-B	1	264.30	264.30	14.46	0.1637
Error	1	18.27	18.27		

Table B.2. ANOVA for relative change in pollen tube length of *Lilium* sp.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	1472.68	1472.68	2954.50	0.0117
Trial	1	42.40	42.40	85.06	0.0688
UV-B	1	97.73	97.73	196.06	0.0454
Error	1	0.50	0.50		

Table B.3. ANOVA for relative change in pollen tube length of *Zigadenus venenosus*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	1964.69	1964.69	271.48	0.0386
Trial	1	8.70	8.70	1.20	0.4707
UV-B	1	22.16	22.16	3.06	0.3305
Error	1	7.24	7.24		

Table B.4. ANOVA for relative change in pollen tube length of *Secale cereale*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	0.04	0.04	205.89	0.0443
Trial	1	0.00	0.00	2.79	0.3436
UV-B	1	0.00	0.00	18.31	0.1461
Error	1	0.00	0.00		

Table B.5. ANOVA for relative change in pollen tube length of *Zea mays* 'Sweet Corn Bodacious Hybrid'.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	798.71	798.71	567.55	0.0267
Trial	1	22.53	22.53	16.01	0.1559
UV-B	1	111.68	111.68	79.36	0.0712
Error	1	1.41	1.41		

Table B.6. ANOVA for relative change in pollen tube length of *Zea mays* 'Popcorn South American Yellow'.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	776.20	776.20	15.10	0.1604
Trial	1	1.51	1.51	0.03	0.8920
UV-B	1	260.60	260.60	5.07	0.2661
Error	1	51.41	51.41		

Table B.7. ANOVA for relative change in pollen tube length of *Tradescantia blossfeldiana*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	1445.53	1445.53	104.14	0.0622
Trial	1	0.11	0.11	0.01	0.9428
UV-B	1	0.60	0.60	0.04	0.8695
Error	1	13.88	13.88		

Table B.8. ANOVA for relative change in pollen tube length of *Malus* sp.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	1677.80	1677.80	260.40	0.0394
Trial	1	2.21	2.21	0.34	0.6625
UV-B	1	123.14	123.14	19.11	0.1432
Error	1	6.44	6.44		

Table B.9. ANOVA for relative change in pollen tube length of *Prunus avium*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	914.63	914.63	24.62	0.1266
Trial	1	24.41	24.41	0.66	0.5663
UV-B	1	16.81	16.81	0.45	0.6230
Error	1	37.14	37.14		

Table B.10. ANOVA for relative change in pollen tube length of *Prunus cerasus*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	637.22	637.22	43.41	0.0959
Trial	1	0.01	0.01	0.00	0.9831
UV-B	1	58.10	58.10	3.96	0.2965
Error	1	14.68	14.68		

Table B.11. ANOVA for relative change in pollen tube length of *Prunus cerasifera*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	932.35	932.35	4817.90	0.0092
Trial	1	0.40	0.40	2.08	0.3856
UV-B	1	4.07	4.07	21.04	0.1367
Error	1	0.19	0.19		

Table B.12. ANOVA for relative change in pollen tube length of *Prunus mahaleb*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	1547.43	1547.43	151.15	0.0517
Trial	1	55.57	55.57	5.43	0.2581
UV-B	1	30.10	30.10	2.94	0.3361
Error	1	10.24	10.24		

Table B.13. ANOVA for relative change in pollen tube length of *Prunus virginiana*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	189.50	189.50	1.40	0.4469
Trial	1	150.88	150.88	1.11	0.4830
UV-B	1	177.27	177.27	1.31	0.4574
Error	1	135.56	135.56		

Table B.14. ANOVA for relative change in pollen tube length of *Pyrus communis*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	927.59	927.59	293.59	0.0371
Trial	1	0.82	0.82	0.26	0.7008
UV-B	1	129.84	129.84	41.10	0.0985
Error	1	3.16	3.16		

Table B.15. ANOVA for relative change in pollen tube length of *Sorbus aucuparia*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	852.38	852.38	133.73	0.0549
Trial	1	19.30	19.30	3.03	0.3321
UV-B	1	30.48	30.48	4.78	0.2731
Error	1	6.37	6.37		

Table B.16. ANOVA for relative change in pollen tube length of *Isatis tictoria*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	449.21	449.21	67.38	0.0772
Trial	1	0.33	0.33	0.05	0.8609
UV-B	1	108.56	108.56	16.28	0.1547
Error	1	6.67	6.67		

Table B.17. ANOVA for relative change in pollen tube length of *Caryopteris clandonensis*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	585.36	585.36	88.71	0.0673
Trial	1	0.78	0.78	0.12	0.7894
UV-B	1	18.94	18.94	2.87	0.34
Error	1	6.60	6.60		

Table B.18. ANOVA for relative change in pollen tube length of *Lonicera periclymenum*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	1210.18	1210.18	215.99	0.0433
Trial	1	25.17	25.17	4.49	0.2807
UV-B	1	13.69	13.69	2.44	0.3623
Error	1	5.60	5.60		

Table B.19. ANOVA for relative change in pollen tube length of *Viburnum lantana*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	491.53	491.53	1.40	0.4463
Trial	1	13.48	13.48	0.04	0.8766
UV-B	1	867.39	867.39	2.48	0.3603
Error	1	350.11	350.11		

Table B.20. ANOVA for relative change in pollen tube length of *Pistacia vera*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	730.90	730.90	227.82	0.0421
Trial	1	0.05	0.05	0.02	0.9194
UV-B	1	9.48	9.48	2.95	0.3355
Error	1	3.21	3.21		

Table B.21. ANOVA for relative change in pollen tube length of *Eschscholzia californicum*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	1047.20	1047.20	3235.54	0.0112
Trial	1	1.67	1.67	5.16	0.2640
UV-B	1	38.08	38.08	117.64	0.0585
Error	1	0.32	0.32		

Table B.22. ANOVA for relative change in pollen tube length of *Papaver somniferum*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	90.88	90.88	10.22	0.1930
Trial	1	39.10	39.10	4.40	0.2833
UV-B	1	18.31	18.31	2.06	0.3875
Error	1	8.89	8.89		

Table B.23. ANOVA for relative change in pollen tube length of *Nicotiana tabacum*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	327.71	327.71	5.83	0.1372
Trial	2	159.87	79. 93	1.42	0.4130
UV-B	1	11.81	11.81	0.21	0.6918
Error	2	112.50	56.25		

Table B.24. ANOVA for relative change in pollen tube length of *Petunia hybrida*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	58.06	58.06	72.80	0.0743
Trial	1	24.51	24.51	30.74	0.1136
UV-B	1	165.79	165.79	207.90	0.0441
Error	1	0.80	0.80		

Table B.25. ANOVA for relative change in pollen tube length of *Oenothera caespitosa*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	825.71	825.71	77.70	0.0719
Trial	1	13.52	13.52	1.27	0.4617
UV-B	1	15.22	15.22	1.43	0.4431
Error	1	10.63	10.63		

Table B.26. ANOVA for relative change in pollen tube length of *Oenothera pallida*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	354.92	354.92	324.20	0.0353
Trial	1	0.00	0.00	0.00	0.9648
UV-B	1	10.96	10.96	10.01	0.1949
Error	1	1.10	1.10		

Table B.27. ANOVA for relative change in pollen tube length of *Epilobium* sp.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	688.23	688.23	8.10	0.2151
Trial	1	0.55	0.55	0.01	0.9487
UV-B	1	101.33	101.33	1.19	0.4720
Error	1	84.94	84.94		

Table B.28. ANOVA for relative change in pollen tube length of *Begonia semperflorens*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	0.84	0.84	0.01	0.9405
Trial	1	0.14	0.14	0.00	0.9757
UV-B	1	21.66	21.66	0.23	0.7163
Error	1	94.96	94.96		

Table B.29. ANOVA for relative change in pollen tube length of *Cleome serrulata*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	666.14	666.14	17.38	0.1499
Trial	1	0.02	0.02	0.00	0.9870
UV-B	1	13.53	13.53	0.35	0.6588
Error	1	38.33	38.33		

Table B.30. ANOVA for relative change in pollen tube length of *Cleome spinosa*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	0.62	0.62	0.01	0.9548
Trial	1	184.75	184.75	1.51	0.4351
UV-B	1	15.77	15.77	0.13	0.7808
Error	1	122.56	122.56		

Table B.31. ANOVA for relative change in pollen tube length of *Nemophila maculata*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	761.15	761.15	2662.38	0.0123
Trial	1	47.49	47.49	166.11	0.0493
UV-B	1	65.33	65.33	228.50	0.0421
Error	1	0.29	0.29		

Table B.32. ANOVA for relative change in pollen tube length of *Phacelia tanacetifolia*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	6.27	6.27	0.03	0.8828
Trial	2	280.93	140.46	0.62	0.6159
UV-B	1	193.53	193.53	0.86	0.4518
Error	2	450.42	225.21		

Table B.33. ANOVA for relative change in pollen germination of *Pistacia vera*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	2.88	2.88	0.00	0.9792
Trial	1	842.78	842.78	0.31	0.6752
UV-B	1	216.69	216.69	0.08	0.8240
Error	1	2690.72	2690.72		

Table B.34. ANOVA for relative change in pollen germination of *Cleome spinosa*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	1069.16	1069.16	5.44	0.2578
Trial	1	22.44	22.44	0.11	0.7925
UV-B	1	1455.06	1455.06	7.41	0.2242
Error	1	196.47	196.47		

Table B.35. ANOVA for relative change in pollen germination of *Lonicera periclymenum*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	899.83	899.83	13.69	0.1680
Trial	1	12.37	12.37	0.19	0.7394
UV-B	1	0.97	0.97	0.01	0.9231
Error	1	65.73	65.73		

Table B.36. ANOVA for relative change in pollen germination of *Viburnum lantana*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	7864.33	7864.33	1.59	0.4271
Trial	1	5391.47	5391.47	1.09	0.4865
UV-B	1	17129.31	17129.31	3.46	0.3141
Error	1	4953.44	4953.44		

Table B.37. ANOVA for relative change in pollen germination of *Tradescantia blossfeldiana*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	16.49	16.49	0.11	0.7945
Trial	1	373.73	373.73	2.54	0.3569
UV-B	1	408.37	408.37	2.77	0.3444
Error	1	147.35	147.35		

Table B.38. ANOVA for relative change in pollen germination of *Nemophila maculata*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	594.40	594.40	0.63	0.5720
Trial	1	498.32	498.32	0.53	0.5991
UV-B	1	371.69	371.69	0.40	0.6424
Error	1	938.13	938.13		

Table B.39. ANOVA for relative change in pollen germination of *Phacelia tanacetifolia*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	236.38	236.38	5.73	0.1390
Trial	2	202.92	101.46	2.46	0.2890
UV-B	1	139.03	139.03	3.37	0.2078
Error	2	82.50	41.25		

Table B.40. ANOVA for relative change in pollen germination of *Allium ostrowskianum*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	255.82	255.82	5.84	0.2499
Trial	1	439.74	439.74	10.03	0.1947
UV-B	1	0.02	0.02	0.00	0.9875
Error	1	43.83	43.83		

Table B.41. ANOVA for relative change in pollen germination of *Lilium* sp.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	944.44	944.44	10.67	0.1891
Trial	1	1.84	1.84	0.02	0.9090
UV-B	1	27.68	27.68	0.31	0.6754
Error	1	88.53	88.53		

Table B.42. ANOVA for relative change in pollen germination of *Zigadenus venenosus*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	998.22	998.22	7.40	0.2243
Trial	1	581.45	581.45	4.31	0.2858
UV-B	1	322.14	322.14	2.39	0.3657
Error	1	134.98	134.98		

Table B.43. ANOVA for relative change in pollen germination of *Oenothera caespitosa*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	248.36	248.36	1027.77	0.0199
Trial	1	3.30	3.30	13.64	0.1683
UV-B	1	1.83	1.83	7.58	0.2218
Error	1	10.63	10.63		

Table B.44. ANOVA for relative change in pollen germination of *Oenothera pallida*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	406.51	406.51	90.19	0.0668
Trial	1	102.53	102.53	22.75	0.1316
UV-B	1	10.09	10.09	2.24	0.3751
Error	1	4.51	4.51		

Table B.45. ANOVA for relative change in pollen germination of *Epilobium* sp.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	1789.00	1789.00	8.99	0.2050
Trial	1	4003.98	4003.98	20.11	0.1397
UV-B	1	0.04	0.04	0.00	0.9908
Error	1	199.10	199.10		

Table B.46. ANOVA for relative change in pollen germination of *Eschscholzia californica*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	85.71	85.71	0.05	0.8639
Trial	1	657.58	657.58	0.36	0.6554
UV-B	1	568.00	568.00	0.31	0.6756
Error	1	1819.30	1819.30		

Table B.47. ANOVA for relative change in pollen germination of *Secale cereale*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	0.00	0.00	0.01	0.9427
Trial	1	0.04	0.04	1.32	0.4561
UV-B	1	0.01	0.01	0.28	0.6906
Error	1	0.03	0.03		

Table B.48. ANOVA for relative change in pollen germination of *Zea mays* (sweet corn).

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	22.77	22.77	12.92	0.1728
Trial	1	0.06	0.06	0.03	0.8864
UV-B	1	431.93	431.93	245.03	0.0406
Error	1	1.76	1.76		

Table B.49. ANOVA for relative change in pollen germination of *Zea mays* (popcorn).

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	1.18	1.18	0.01	0.9351
Trial	1	1.19	1.19	0.01	0.9348
UV-B	1	5.55	5.55	0.05	0.8609
Error	1	112.62	112.62		

Table B.50. ANOVA for relative change in pollen germination of *Malus* sp.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	2301.10	2301.10	2.10	0.3843
Trial	1	62.06	62.06	0.06	0.8512
UV-B	1	5150.98	5150.98	4.71	0.2749
Error	1	1094.04	1094.04		

Table B.51. ANOVA for relative change in pollen germination of *Prunus avium*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	2.79	2.79	0.02	0.9085
Trial	1	52.34	52.34	0.39	0.6437
UV-B	1	203.12	203.12	1.52	0.4335
Error	1	133.36	133.36		

Table B.52. ANOVA for relative change in pollen germination of *Prunus cerasifera*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	791.05	791.05	222.99	0.0426
Trial	1	42.65	42.65	12.02	0.1788
UV-B	1	4.30	4.30	1.21	0.4695
Error	1	3.55	3.55		

Table B.53. ANOVA for relative change in pollen germination of *Prunus cerasus*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	255.07	255.07	82.62	0.0698
Trial	1	95.35	95.35	30.88	0.1133
UV-B	1	144.19	144.19	46.71	0.0925
Error	1	3.09	3.09		

Table B.54. ANOVA for relative change in pollen germination of *Prunus mahaleb*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	16.66	16.66	0.13	0.7769
Trial	1	5.18	5.18	0.04	0.8721
UV-B	1	199.03	199.03	1.60	0.4262
Error	1	124.69	124.69		

Table B.55. ANOVA for relative change in pollen germination of *Prunus virginiana*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	147.26	147.26	1.16	0.4757
Trial	1	177.20	177.20	1.40	0.4465
UV-B	1	0.00	0.00	0.00	0.9982
Error	1	126.42	126.42		

Table B.56. ANOVA for relative change in pollen germination of *Pyrus communis*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	552.94	552.94	54.76	0.0855
Trial	1	9.19	9.19	0.91	0.5149
UV-B	1	140.84	140.84	13.95	0.1666
Error	1	10.10	10.10		

Table B.57. ANOVA for relative change in pollen germination of *Sorbus aucuparia*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	70.60	70.60	21.85	0.1342
Trial	1	164.60	164.60	50.95	0.0886
UV-B	1	37.66	37.66	11.66	0.1814
Error	1	3.23	3.23		

Table B.58. ANOVA for relative change in pollen germination of *Nicotiana tabacum*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	537.87	537.87	0.73	0.4830
Trial	2	1723.63	861.82	1.17	0.4611
UV-B	1	164.71	164.71	0.22	0.6830
Error	2	1474.61	737.30		

Table B.59. ANOVA for relative change in pollen germination of *Caryopteris clandonensis*.

Source of variation	df	SS	MS	F-value	*P*-value
Intercept	1	8.75	8.75	0.11	0.7988
Trial	1	225.77	225.77	2.76	0.3451
UV-B	1	3.90	3.90	0.05	0.8633
Error	1	81.88	81.88		

Table B.60. ANOVA for height of parents and hybrids.

Source of variation	df	SS	MS	F-value	*P*-value
Genotype (G)	27	13989.78	518.14	92.42	0.0001
Treatment (T)	1	1142.15	1142.15	203.73	0.0001
G X T	27	475.14	17.60	3.14	0.0020
Error	27	151.37	5.61		

Table B.61. ANOVA for number of shoots of parents and hybrids.

Source of variation	df	SS	MS	F-value	*P*-value
Genotype (G)	27	1297.07	48.04	33.12	0.0001
Treatment (T)	1	4.68	4.68	3.22	0.0838
G X T	27	39.37	1.46	1.01	0.4946
Error	27	39.17	1.45		

Table B.62. ANOVA for number of branches of parents and hybrids.

Source of variation	df	SS	MS	F-value	*P*-value
Genotype (G)	27	110.30	4.09	3.97	0.0003
Treatment (T)	1	0.66	0.66	0.64	0.4315
G X T	27	14.46	0.54	0.52	0.9518
Error	27	27.75	1.03		

Table B.63. ANOVA for rosette diameter of parents and hybrids.

Source of variation	df	SS	MS	F-value	*P*-value
Genotype (G)	27	1458.26	54.01	185.05	0.0001
Treatment (T)	1	7.73	7.73	26.49	0.0001
G X T	27	17.06	0.63	2.17	0.0247
Error	27	7.88	0.29		

Table B.64. ANOVA for vegetative dry mass of parents and hybrids.

Source of variation	df	SS	MS	F-value	*P*-value
Genotype (G)	27	14.64	0.54	27.93	0.0001
Treatment (T)	1	0.96	0.96	49.46	0.0001
G X T	27	1.13	0.04	2.16	0.0252
Error	27	0.52	0.02		

Table B.65. ANOVA for reproductive dry mass of parents and hybrids.

Source of variation	df	SS	MS	F-value	*P*-value
Genotype (G)	27	36.68	1.36	71.80	0.0001
Treatment (T)	1	0.32	0.32	16.68	0.0004
G X T	27	0.93	0.04	1.83	0.0620
Error	27	0.51	0.02		

Table B.66. F-tests for height, based on fixed model for GCA and SCA.

Source of variation	df	MS	F-value	*P*-value
GCA	6	85.01	32.43	0.0000
SCA	21	20.97	1.53	0.1672
Error	27	13.67		

Table B.67. F-tests for number of shoots, based on fixed model for GCA and SCA.

Source of variation	df	MS	F-value	*P*-value
GCA	6	2.79	0.60	0.7257
SCA	21	2.95	1.23	0.3217
Error	27	2.41		

Table B.68. F-tests for number of branches, based on fixed model for GCA and SCA.

Source of variation	df	MS	F-value	*P*-value
GCA	6	0.94	0.28	0.9403
SCA	21	1.11	0.66	0.8256
Error	27	1.68		

Table B.69. F-tests for rosette diameter, based on fixed model for GCA and SCA.

Source of variation	df	MS	F-value	*P*-value
GCA	6	0.73	1.18	0.3570
SCA	21	1.42	2.47	0.0220
Error	27	0.57		

Table B.70. F-tests for vegetative dry mass, based on fixed model for GCA and SCA.

Source of variation	df	MS	F-value	*P*-value
GCA	6	0.82	2.49	0.0563
SCA	21	0.84	2.08	0.0506
Error	27	0.41		

Table B.71. F-tests for reproductive dry mass, based on fixed model for GCA and SCA.

Source of variation	df	MS	F-value	*P*-value
GCA	6	0.12	0.39	0.8755
SCA	21	0.56	1.39	0.2299
Error	27	0.40		

Table B.72. ANOVA for height.

Source of variation	Num-df	Den-df	F-value	*P*-value
Post	1	4	150.17	0.0003
Eco	2	44	7.29	0.0018
Post X Eco	2	44	2.27	0.1148
Gen	1	44	9.67	0.0033
Post X Gen	1	44	0.02	0.8829
Eco X Gen	2	44	9.27	0.0004
Post X Eco X Gen	2	44	2.90	0.0656
UV-B	1	44	0.13	0.7208
Post X UV-B	1	44	0.28	0.6010
Eco X UV-B	2	44	0.53	0.5898
Post X Eco X UV-B	2	44	0.09	0.9176
Gen X UV-B	1	44	1.84	0.1820
Post X Gen X UV-B	1	44	4.34	0.0430
Eco X Gen X UV-B	2	44	1.42	0.2521
Post X Eco X Gen X UV-B	2	44	8.69	0.0007

Covariance estimates:

Covariance Parameter	Estimate	Std Error	Z-value	*P*-value
Rep (Post)	0.5704	0.5099	1.12	0.2633
Residual	1.7917	0.3820	4.69	0.0001

Table B.73. ANOVA for number of shoots.

Source of variation	Num-df	Den-df	F-value	*P*-value
Post	1	4	0.02	0.8891
Eco	2	44	88.17	0.0001
Post X Eco	2	44	0.19	0.8281
Gen	1	44	2.10	0.1541
Post X Gen	1	44	0.86	0.3595
Eco X Gen	2	44	20.85	0.0001
Post X Eco X Gen	2	44	0.35	0.7085
UV-B	1	44	0.92	0.3430
Post X UV-B	1	44	1.12	0.2963
Eco X UV-B	2	44	38.77	0.0001
Post X Eco X UV-B	2	44	1.46	0.2438
Gen X UV-B	1	44	0.40	0.5313
Post X Gen X UV-B	1	44	0.00	0.9804
Eco X Gen X UV-B	2	44	17.42	0.0001
Post X Eco X Gen X UV-B	2	44	0.06	0.9390

Covariance estimates:

Covariance Parameter	Estimate	Std Error	Z-value	*P*-value
Rep (Post)	1.4190	1.0883	1.30	0.1923
Residual	1.4365	0.3063	4.69	0.0001

Table B.74. ANOVA for number of branches.

Source of variation	Num-df	Den-df	F-value	*P*-value
Post	1	4	3.51	0.1344
Eco	2	44	9.28	0.0004
Post X Eco	2	44	0.45	0.6388
Gen	1	44	1.37	0.2488
Post X Gen	1	44	0.28	0.5979
Eco X Gen	2	44	8.20	0.0009
Post X Eco X Gen	2	44	1.09	0.3458
UV-B	1	44	0.25	0.6224
Post X UV-B	1	44	0.85	0.3621
Eco X UV-B	2	44	3.20	0.0506
Post X Eco X UV-B	2	44	4.75	0.0136
Gen X UV-B	1	44	1.28	0.2632
Post X Gen X UV-B	1	44	0.25	0.6224
Eco X Gen X UV-B	2	44	2.71	0.0776
Post X Eco X Gen X UV-B	2	44	0.91	0.4107

Covariance estimates:

Covariance Parameter	Estimate	Std Error	Z-value	*P*-value
Rep (Post)	0.0914	0.0829	1.10	0.2705
Residual	0.3076	0.0656	4.69	0.0001

Table B.75. ANOVA for rosette diameter.

Source of variation	Num-df	Den-df	F-value	*P*-value
Post	1	4	1.45	0.2953
Eco	2	44	0.53	0.5950
Post X Eco	2	44	0.32	0.7250
Gen	1	44	5.79	0.0204
Post X Gen	1	44	0.02	0.8949
Eco X Gen	2	44	0.57	0.5714
Post X Eco X Gen	2	44	0.35	0.7068
UV-B	1	44	0.40	0.5317
Post X UV-B	1	44	0.64	0.4274
Eco X UV-B	2	44	1.19	0.3128
Post X Eco X UV-B	2	44	0.28	0.7606
Gen X UV-B	1	44	0.23	0.6362
Post X Gen X UV-B	1	44	0.20	0.6541
Eco X Gen X UV-B	2	44	4.79	0.0131
Post X Eco X Gen X UV-B	2	44	0.79	0.4619

Covariance estimates:

Covariance Parameter	Estimate	Std Error	Z-value	*P*-value
Rep (Post)	0.0945	0.1237	0.76	0.4449
Residual	0.9456	0.2016	4.69	0.0001

Table B.76. ANOVA for vegetative dry mass.

Source of variation	Num-df	Den-df	F-value	*P*-value
Post	1	4	11.93	0.0260
Eco	2	44	1.50	0.2340
Post X Eco	2	44	0.86	0.4293
Gen	1	44	0.64	0.4288
Post X Gen	1	44	0.13	0.7164
Eco X Gen	2	44	0.76	0.4737
Post X Eco X Gen	2	44	0.07	0.9355
UV-B	1	44	0.57	0.4541
Post X UV-B	1	44	0.00	0.9845
Eco X UV-B	2	44	1.70	0.1947
Post X Eco X UV-B	2	44	1.14	0.3284
Gen X UV-B	1	44	1.15	0.2898
Post X Gen X UV-B	1	44	1.96	0.1686
Eco X Gen X UV-B	2	44	5.36	0.0083
Post X Eco X Gen X UV-B	2	44	0.33	0.7231

Covariance estimates:

Covariance Parameter	Estimate	Std Error	Z-value	*P*-value
Rep (Post)	0.0017	0.0027	0.64	0.5233
Residual	0.0247	0.0053	4.69	0.0001

Table B.77. ANOVA for reproductive dry mass.

Source of variation	Num-df	Den-df	F-value	*P*-value
Post	1	4	9.06	0.0396
Eco	2	44	2.02	0.1447
Post X Eco	2	44	1.57	0.2203
Gen	1	44	3.73	0.0598
Post X Gen	1	44	0.18	0.6765
Eco X Gen	2	44	0.96	0.3901
Post X Eco X Gen	2	44	0.13	0.8779
UV-B	1	44	0.12	0.7292
Post X UV-B	1	44	0.03	0.8651
Eco X UV-B	2	44	2.01	0.1457
Post X Eco X UV-B	2	44	1.30	0.2817
Gen X UV-B	1	44	1.39	0.2447
Post X Gen X UV-B	1	44	2.78	0.1024
Eco X Gen X UV-B	2	44	4.90	0.0120
Post X Eco X Gen X UV-B	2	44	0.21	0.8122

Covariance estimates:

Covariance Parameter	Estimate	Std Error	Z-value	*P*-value
Rep (Post)	0.0031	0.0031	0.99	0.3199
Residual	0.0156	0.0033	4.69	0.0001

Table B.78. ANOVA for ratio of reproductive dry mass/vegetative dry mass.

Source of variation	Num-df	Den-df	F-value	*P*-value
Post	1	4	3.41	0.1383
Eco	2	44	5.78	0.0059
Post X Eco	2	44	2.79	0.0726
Gen	1	44	35.57	0.0001
Post X Gen	1	44	0.09	0.7617
Eco X Gen	2	44	0.46	0.6354
Post X Eco X Gen	2	44	0.49	0.6164
UV-B	1	44	3.25	0.0781
Post X UV-B	1	44	0.10	0.7550
Eco X UV-B	2	44	1.98	0.1499
Post X Eco X UV-B	2	44	0.12	0.8864
Gen X UV-B	1	44	0.11	0.7427
Post X Gen X UV-B	1	44	0.75	0.3925
Eco X Gen X UV-B	2	44	0.63	0.5378
Post X Eco X Gen X UV-B	2	44	1.21	0.3082

Covariance estimates:

Covariance Parameter	Estimate	Std Error	Z-value	*P*-value
Rep (Post)	2.2085	1.8325	1.21	0.2281
Residual	4.5651	0.9733	4.69	0.0001

Table B.79. ANOVA for specific leaf mass.

Source of variation	Num-df	Den-df	F-value	*P*-value
Post	1	4	0.95	0.3848
Eco	2	44	5.61	0.0067
Post X Eco	2	44	0.56	0.5755
Gen	1	44	0.83	0.3671
Post X Gen	1	44	2.79	0.1018
Eco X Gen	2	44	3.48	0.0396
Post X Eco X Gen	2	44	0.18	0.8369
UV-B	1	44	2.79	0.1018
Post X UV-B	1	44	2.31	0.1359
Eco X UV-B	2	44	8.49	0.0008
Post X Eco X UV-B	2	44	0.25	0.7814
Gen X UV-B	1	44	0.21	0.6509
Post X Gen X UV-B	1	44	0.83	0.3671
Eco X Gen X UV-B	2	44	6.90	0.0025
Post X Eco X Gen X UV-B	2	44	1.57	0.2185

Covariance estimates:

Covariance Parameter	Estimate	Std Error	Z-value	*P*-value
Rep (Post)	0.0000	.	.	.
Residual	0.0000	.	.	.

Table B.80. ANOVA for days to flowering.

Source of variation	Num-df	Den-df	F-value	*P*-value
Post	1	4	3.37	0.1405
Eco	2	44	69.42	0.0001
Post X Eco	2	44	3.17	0.0516
Gen	1	44	40.70	0.0001
Post X Gen	1	44	0.01	0.9247
Eco X Gen	2	44	49.45	0.0001
Post X Eco X Gen	2	44	0.39	0.6774
UV-B	1	44	4.31	0.0438
Post X UV-B	1	44	0.23	0.6363
Eco X UV-B	2	44	27.02	0.0001
Post X Eco X UV-B	2	44	0.04	0.9602
Gen X UV-B	1	44	0.16	0.6912
Post X Gen X UV-B	1	44	0.39	0.5330
Eco X Gen X UV-B	2	44	37.85	0.0001
Post X Eco X Gen X UV-B	2	44	0.56	0.5745

Covariance estimates:

Covariance Parameter	Estimate	Std Error	Z-value	*P*-value
Rep (Post)	0.0214	0.0311	0.69	0.4927
Residual	0.2660	0.0567	4.69	0.0001

Table B.81. ANOVA for chlorophyll (mg m^{-2}).

Source of variation	Num-df	Den-df	F-value	*P*-value
Post	1	4	81.11	0.0008
Eco	2	44	3.48	0.0395
Post X Eco	2	44	0.65	0.5267
Gen	1	44	3.56	0.0658
Post X Gen	1	44	2.67	0.1091
Eco X Gen	2	44	5.73	0.0061
Post X Eco X Gen	2	44	0.99	0.3811
UV-B	1	44	0.51	0.4804
Post X UV-B	1	44	3.69	0.0613
Eco X UV-B	2	44	1.64	0.2056
Post X Eco X UV-B	2	44	1.02	0.3696
Gen X UV-B	1	44	0.71	0.4024
Post X Gen X UV-B	1	44	1.34	0.2525
Eco X Gen X UV-B	2	44	2.53	0.0915
Post X Eco X Gen X UV-B	2	44	3.32	0.0454

Covariance estimates:

Covariance Parameter	Estimate	Std Error	Z-value	*P*-value
Rep (Post)	0.7936	0.6895	1.15	0.2497
Residual	2.1595	0.4604	4.69	0.0001

Table B.82. ANOVA for chlorophyll (mg g^{-1}).

Source of variation	Num-df	Den-df	F-value	*P*-value
Post	1	4	0.93	0.3897
Eco	2	44	2.94	0.0632
Post X Eco	2	44	2.01	0.1459
Gen	1	44	0.12	0.7323
Post X Gen	1	44	4.33	0.0432
Eco X Gen	2	44	1.50	0.2344
Post X Eco X Gen	2	44	0.91	0.4090
UV-B	1	44	0.98	0.3269
Post X UV-B	1	44	0.83	0.3686
Eco X UV-B	2	44	6.28	0.0040
Post X Eco X UV-B	2	44	0.55	0.5788
Gen X UV-B	1	44	0.19	0.6650
Post X Gen X UV-B	1	44	0.28	0.6025
Eco X Gen X UV-B	2	44	8.22	0.0009
Post X Eco X Gen X UV-B	2	44	0.25	0.7790

Covariance estimates:

Covariance Parameter	Estimate	Std Error	Z-value	*P*-value
Rep (Post)	140.8401	103.0770	1.37	0.1718
Residual	59.1025	12.6007	4.69	0.0001

Table B.83. ANOVA for absorbance reading at 260 nm.

Source of variation	Num-df	Den-df	F-value	*P*-value
Post	1	4	7.24	0.0547
Eco	2	44	17.61	0.0001
Post X Eco	2	44	0.07	0.9357
Gen	1	44	0.17	0.6828
Post X Gen	1	44	1.57	0.2164
Eco X Gen	2	44	8.96	0.0005
Post X Eco X Gen	2	44	2.49	0.0944
UV-B	1	44	0.04	0.8458
Post X UV-B	1	44	4.93	0.0315
Eco X UV-B	2	44	9.72	0.0003
Post X Eco X UV-B	2	44	0.59	0.5613
Gen X UV-B	1	44	2.85	0.0986
Post X Gen X UV-B	1	44	1.43	0.2375
Eco X Gen X UV-B	2	44	12.52	0.0001
Post X Eco X Gen X UV-B	2	44	2.86	0.0679

Covariance estimates:

Covariance Parameter	Estimate	Std Error	Z-value	*P*-value
Rep (Post)	5339.9077	3901.9364	1.37	0.1711
Residual	2136.0317	455.4035	4.69	0.0001

Table B.84. ANOVA for absorbance reading at 305 nm.

Source of variation	Num-df	Den-df	F-value	*P*-value
Post	1	4	7.15	0.0556
Eco	2	44	16.70	0.0001
Post X Eco	2	44	2.13	0.1304
Gen	1	44	0.17	0.6829
Post X Gen	1	44	2.49	0.1217
Eco X Gen	2	44	10.33	0.0002
Post X Eco X Gen	2	44	1.18	0.3177
UV-B	1	44	0.04	0.8441
Post X UV-B	1	44	1.66	0.2050
Eco X UV-B	2	44	9.88	0.0003
Post X Eco X UV-B	2	44	0.45	0.6423
Gen X UV-B	1	44	1.78	0.1896
Post X Gen X UV-B	1	44	1.15	0.2886
Eco X Gen X UV-B	2	44	8.13	0.0010
Post X Eco X Gen X UV-B	2	44	1.50	0.2350

Covariance estimates:

Covariance Parameter	Estimate	Std Error	Z-value	*P*-value
Rep (Post)	1906.6815	1443.1766	1.32	0.1864
Residual	1606.5502	342.5177	4.69	0.0001

Table B.85. ANOVA for absorbance reading at 320 nm.

Source of variation	Num-df	Den-df	F-value	*P*-value
Post	1	4	10.24	0.0329
Eco	2	44	14.29	0.0001
Post X Eco	2	44	1.66	0.2024
Gen	1	44	0.23	0.6349
Post X Gen	1	44	3.02	0.0895
Eco X Gen	2	44	8.95	0.0005
Post X Eco X Gen	2	44	1.02	0.3678
UV-B	1	44	0.02	0.8845
Post X UV-B	1	44	1.40	0.2435
Eco X UV-B	2	44	7.48	0.0016
Post X Eco X UV-B	2	44	0.16	0.8502
Gen X UV-B	1	44	1.38	0.2462
Post X Gen X UV-B	1	44	1.22	0.2761
Eco X Gen X UV-B	2	44	7.04	0.0022
Post X Eco X Gen X UV-B	2	44	1.61	0.2123

Covariance estimates:

Covariance Parameter	Estimate	Std Error	Z-value	*P*-value
Rep (Post)	2119.7363	1615.6577	1.31	0.1895
Residual	1975.3155	421.1387	4.69	0.0001

Table B.86. ANOVA for absorbance reading at 355 nm.

Source of variation	Num-df	Den-df	F-value	*P*-value
Post	1	4	19.33	0.0117
Eco	2	44	11.26	0.0001
Post X Eco	2	44	1.04	0.3611
Gen	1	44	0.21	0.6505
Post X Gen	1	44	2.29	0.1375
Eco X Gen	2	44	6.24	0.0041
Post X Eco X Gen	2	44	0.74	0.4843
UV-B	1	44	0.15	0.7034
Post X UV-B	1	44	1.24	0.2709
Eco X UV-B	2	44	4.29	0.0199
Post X Eco X UV-B	2	44	0.17	0.8414
Gen X UV-B	1	44	1.74	0.1942
Post X Gen X UV-B	1	44	0.90	0.3482
Eco X Gen X UV-B	2	44	5.11	0.0101
Post X Eco X Gen X UV-B	2	44	1.79	0.1797

Covariance estimates:

Covariance Parameter	Estimate	Std Error	Z-value	*P*-value
Rep (Post)	2412.8008	1837.2752	1.31	0.1891
Residual	2218.8067	473.0512	4.69	0.0001

Table B.87. ANOVA for absorbance reading at 398 nm.

Source of variation	Num-df	Den-df	F-value	*P*-value
Post	1	4	1.91	0.2393
Eco	2	44	8.81	0.0006
Post X Eco	2	44	1.03	0.3648
Gen	1	44	1.38	0.2460
Post X Gen	1	44	1.89	0.1758
Eco X Gen	2	44	3.75	0.0313
Post X Eco X Gen	2	44	0.80	0.4568
UV-B	1	44	0.63	0.4332
Post X UV-B	1	44	2.39	0.1289
Eco X UV-B	2	44	9.56	0.0004
Post X Eco X UV-B	2	44	0.74	0.4844
Gen X UV-B	1	44	0.38	0.5388
Post X Gen X UV-B	1	44	0.66	0.4204
Eco X Gen X UV-B	2	44	12.74	0.0001
Post X Eco X Gen X UV-B	2	44	0.31	0.7385

Covariance estimates:

Covariance Parameter	Estimate	Std Error	Z-value	*P*-value
Rep (Post)	5517.7645	3998.5426	1.38	0.1676
Residual	1642.5371	350.1901	4.69	0.0001

Table B.88. ANOVA for absorbance reading at 531 nm.

Source of variation	Num-df	Den-df	F-value	*P*-value
Post	1	4	0.06	0.8221
Eco	2	44	18.25	0.0001
Post X Eco	2	44	0.61	0.5484
Gen	1	44	0.39	0.5367
Post X Gen	1	44	0.00	0.9879
Eco X Gen	2	44	7.76	0.0013
Post X Eco X Gen	2	44	0.78	0.4642
UV-B	1	44	0.73	0.3960
Post X UV-B	1	44	0.07	0.7999
Eco X UV-B	2	44	15.46	0.0001
Post X Eco X UV-B	2	44	0.96	0.3920
Gen X UV-B	1	44	0.05	0.8309
Post X Gen X UV-B	1	44	0.00	0.9787
Eco X Gen X UV-B	2	44	16.18	0.0001
Post X Eco X Gen X UV-B	2	44	2.13	0.1309

Covariance estimates:

Covariance Parameter	Estimate	Std Error	Z-value	*P*-value
Rep (Post)	26.0934	21.0262	1.24	0.2146
Residual	43.4653	9.2668	4.69	0.0001

Table B.89. ANOVA for absorbance reading at 654 nm.

Source of variation	Num-df	Den-df	F-value	*P*-value
Post	1	4	0.44	0.5418
Eco	2	44	4.96	0.0114
Post X Eco	2	44	0.57	0.5711
Gen	1	44	1.51	0.2264
Post X Gen	1	44	1.43	0.2385
Eco X Gen	2	44	2.66	0.0813
Post X Eco X Gen	2	44	0.39	0.6765
UV-B	1	44	0.99	0.3257
Post X UV-B	1	44	1.92	0.1728
Eco X UV-B	2	44	6.87	0.0025
Post X Eco X UV-B	2	44	1.09	0.3453
Gen X UV-B	1	44	0.01	0.9077
Post X Gen X UV-B	1	44	0.90	0.3483
Eco X Gen X UV-B	2	44	12.15	0.0001
Post X Eco X Gen X UV-B	2	44	0.07	0.9302

Covariance estimates:

Covariance Parameter	Estimate	Std Error	Z-value	*P*-value
Rep (Post)	1006.9527	732.5660	1.37	0.1693
Residual	348.1815	74.2326	4.69	0.0001

CURRICULUM VITAE

Javad Torabinejad
(May 1999)

EDUCATION:

Ph.D. in Range Ecology, Utah State University, Logan, Utah (expected 6/99). Emphasis in Range Ecology (physiological ecology). MS in Plant Science, Utah State University, Logan, Utah (12/87). Emphasis in Agronomy (plant breeding and cytotaxonomy). BS in Plant Science, Utah State University, Logan, Utah (6/83). Emphasis in Horticulture (fruits and vegetables).

AREAS OF KNOWLEDGE:

Physiological ecology, stress physiology, plant breeding, genetics, cytotaxonomy, plant tissue culture, and greenhouse management.

ACHIEVEMENTS IN RESEARCH:

Obtaining difficult interspecific and intergeneric hybrids involving Australian *Elymus* species; obtaining the first hybrid between an apomictic species in Triticeae and a cereal species, barley; resolving the genomic composition of the Australian hexaploid *Elymus* species; studying the effects of the ultraviolet-B radiation on plant reproductive system; studying the adaptation of *Arabidopsis* to high UV-B environment, including the inheritance of its tolerance; identifying pollination-induced burst of ethylene in wheat; documenting the riboflavin excretion from isolated roots of pepper and squash; and developing a modified white medium for root culture and hybrid embryos.

PUBLICATIONS:

Torabinejad, J. and M. M. Caldwell. Inheritance of UV-B tolerance in seven ecotypes of *Arabidopsis thaliana* and their F1 hybrids. (Submitted)

Torabinejad, J., M. Caldwell, S. D. Flint, S. Durham. 1998. Susceptibility of pollen to UV-B radiation: an assay of 34 taxa. American Journal of Botany 85: 360-369.

Torabinejad, J. and R. J. Mueller. 1993. Genome analysis of intergeneric hybrids of apomictic and sexual Australian *Elymus* species with wheat, barley,

and rye: implication for the transfer of apomixis to cereals. Theor. Appl Genet 86:288-294.

Torabinejad, J. and R. J. Mueller. 1993. Genome constitution of the Australian hexaploid grass *Elymus scabrus* (Poaceae: Triticeae). Genome 36:147-151.

Torabinejad, J., J. G. Carman, and C. F. Crane. 1987. Morphology and genome analysis of interspecific hybrids of *Elymus scabrus*. Genome 29:150-155.

Torabinejad, J. 1987. Interspecific hybridization involving *Elymus scabrus*: Cytology of F1 hybrids and amphiploids. Utah State University. 89pp. (M.S. Thesis).